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•REVIEW•

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The landscape of aging

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Aging is characterized by a progressive deterioration of physiological integrity, leading to impaired functional ability and ultimately increased susceptibility to death. It is a major risk factor for chronic human diseases, including cardiovascular disease, diabetes, neurological degeneration, and cancer. Therefore, the growing emphasis on "healthy aging" raises a series of important questions in life and social sciences. In recent years, there has been unprecedented progress in aging research, particularly the discovery that the rate of aging is at least partly controlled by evolutionarily conserved genetic pathways and biological processes. In an attempt to bring full-fledged understanding to both the aging process and age-associated diseases, we review the descriptive, conceptual, and interventive aspects of the landscape of aging composed of a number of layers at the cellular, tissue, organ, organ system, and organismal levels.

aging, mechanism, intervention

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Introduction

Aging in humans is an incredibly complex process characterized by time-dependent functional decline, resulting in a decrease in the quality of life (Leidal et al., 2018). As medical technology advances, the average lifespan has greatly increased globally. However, coupled with a declining birth rate, it has resulted in an acceleration of population aging. Indeed, the global population over 65 is growing faster than any other age group. Moreover, with this trend, the United Nations (UN) predicts that by 2050, one in six people will be over the age of 65, and the number of people over 80 will triple. Aging is a predominant risk factor for most chronic diseases in humans, including cardiovascular diseases, cancer, and neurodegenerative diseases (Kennedy et al., 2014), and currently, the "healthspan" has not kept up with the increasing lifespan. Aging will be a formidable global socioeconomic burden and a significant healthcare challenge (Scott et al., 2021). Therefore, it is of paramount importance to promote "healthy aging" - the maintenance of

functional ability into older age, by slowing down the progression of age-related pathological conditions in a rapidly aging population (López-Otín and Kroemer, 2021; Partridge et al., 2018).

The landscape of aging is composed of several layers at the cellular, tissue, organ, organ system, and organismal levels (Figure 1). At the very bottom, many hallmarks of aging have been associated with and attributed to senescence, a permanent arrest of cell proliferation, first reported by Hayflick and Moorhead in the early 1960s (Hayflick, 1965; Hayflick and Moorhead, 1961). Stem cells on their route to senescence or stem cell aging are considered one of the major drivers of systemic aging, which impairs both tissue functionality and regeneration capacity, thus leading to age-related disorders (López-Otín et al., 2013). During aging, stem cells accumulate DNA damage, experience epigenetic changes, suffer from dysregulated autophagy and metabolism, and demonstrate senescence and senescence-associated secretory phenotype (SASP), all of which lead to stem cell dysfunction and exhaustion (Aman et al., 2021; Bell et al., 2019; García-Prat et al., 2016; Li et al., 2016a; López-Otín et al., 2013; Pouikli et al., 2021; Rübe et al., 2011). Among these molecular alterations, extensive epigenetic changes occur during senescence, including reduced bulk levels of the core histones, altered patterns of histone modifications and DNA methylation, accounting for a fundamental aspect of the "aging memories" while cross-talking with other aspects (Zhang et al., 2020c). For example, large-scale chromatin reorganization including heterochromatin erosion is an intrinsic and conserved feature of cellular senescence, and reactivates transposable elements that should be tightly sealed and suppressed by heterochromatin. In turn, transposon activation elicits genome instability and dysregulation of gene expression in the nucleus, and triggers inflammatory pathways in the cytosol, where its effects may ripple through mitochondria and induce metabolic dysregulation (Bi et al., 2020; De Cecco et al., 2019; Hagan et al., 2003; Hu et al., 2020; Pal and Tyler, 2016; Rudin and Thompson, 2001). Thus, rejuvenation of an aged cell often involves the modulation of aging memories. Overall, in the first chapter, different aspects of cellular aging memories and the underlying mechanisms will be presented, providing potential targets for rejuvenation and regeneration.

From the bottom up through tissue, organ to organ system

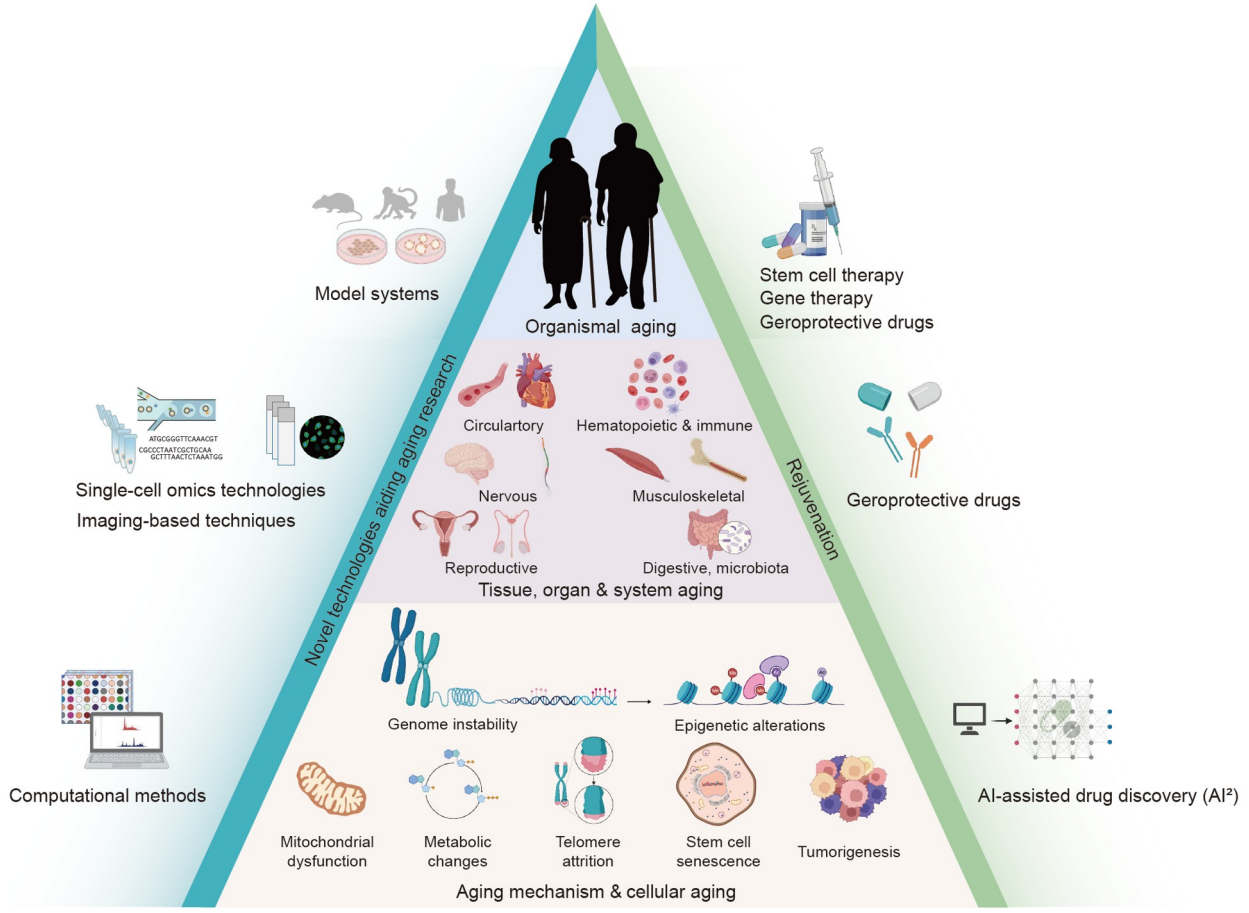

Figure 1 The landscape of aging. A diagram depicting the landscape of aging through layers at the cellular (bottom), tissue, organ & system (middle), organismal levels (top), along with novel technologies aiding aging research (left) and the development of intervention and rejuvenation strategies (right). (Created with BioRender.com).

level, our body systems age at different rates, demonstrating variable molecular features and clinical symptoms, or "ageotypes". Here in the second chapter, we present an in-depth analysis of molecular markers and potential drivers, along with age-associated dysfunction and disorders across tissues and organs in the circulatory system, hematopoietic and immune system, nervous system, musculoskeletal system, reproductive system, digestive system as well as the microbiota therein. Both common and tissue-specific ageotypes exist, and various studies have focused on targeting specific organs and systems to delay aging (He et al., 2020b; Pluvinage and Wyss-Coray, 2020; Simon et al., 2019; Tosato et al., 2007). Moreover, although each organ and system has its own combination of molecular drivers of aging, they are impacted by the systemic milieu as well. The molecular connection between the aging systems and systemic factors that can mediate rejuvenation throughout the body is just beginning to be revealed and has quickly attracted attention from academia to industry.

These understandings of the biological basis of aging and age-related diseases open the door to interventions. Over the past decade, scientists have made great efforts to develop drugs for aging intervention, aiming to ameliorate age-related diseases. For instance, at the forefront, there is growing evidence that eliminating senescence hallmarks can lead to extended healthspan in animal models, which has prompted researchers to develop strategies to mitigate senescence pathways or to wipe out senescent cells themselves, using small molecules or antibodies as senolytic drugs (Baker et al., 2011; Chang et al., 2016). In addition, changes in diet and lifestyle, including a ketogenic diet, calorie restriction, and an appropriate increase in physical activities, are being explored to delay aging (Partridge et al., 2018). Nowadays, state-of-the-art technologies are incorporated into aging research, including new model systems, single-cell omics, bioimaging, and computational analysis. These cumulative approaches provide us with opportunities to understand the intricate process of aging on multiple scales, from single molecule to whole organism, promoting breakthroughs in Geriatrics and Gerontology.

Mechanisms of aging

At the cellular level, many hallmarks of aging have been associated with and attributed to the impairment and exhaustion of stem cells (López-Otín et al., 2013). In addition to stem cell aging, cellular senescence in many cell types is also the main cause of aging, exemplified by a panel of phenotypes from various intracellular and intercellular aspects. In this chapter, we illustrate the breakthrough discoveries in the molecular mechanisms of cellular aging and its alleviation, with the aim of providing a foundation for healthy aging at the very bottom of the landscape of aging.

Stem cell aging

Adult or tissue stem cells, which reside in multiple tissues, can self-renew, and differentiate into cell types that replenish and repair tissues and organs, thereby performing a critical role in tissue homeostasis and regeneration (Oh et al., 2014; Ren et al., 2017). Tissue stem cells are multipotent or unipotent and include hematopoietic stem cells (HSCs), intestinal stem cells (ISCs), muscle stem cells (MuSCs), neural stem cells (NSCs), mesenchymal stem cells (MSCs), and hair follicle stem cells (HFSCs), among others (Cai et al., 2022; Schultz and Sinclair, 2016). These stem cells present in specific, localized tissue microenvironments, also known as "stem cell niches", which maintain homeostasis and plasticity. For tissues with continuously high turnover rates, such as the hematopoietic system and small intestine, at least a portion of tissue stem cells remain activated to proliferate and differentiate into cell types that are required to maintain normal tissue function and structure. Conversely, tissues with a low turnover rate, such as muscles and the brain, contain tissue stem cells that enter a quiescent state and activate to reenter the cell cycle only when injured or stimulated by other environmental stimuli (Schultz and Sinclair, 2016; Zhang et al., 2020c). Consequently, despite an overall small number of tissue stem cells, they play a significant role to ensure the homeostasis of both tissues and the overall organism, and in preventing tissue and organ aging (Sameri et al., 2020; Wang et al., 2016).

However, due to their life-long existence within the body, tissue stem cells are prone to accumulate cellular damage, which eventually contributes to their senescence/aging or loss of function (Schultz and Sinclair, 2016). Whether stem cell aging is the cause or consequence of organismal aging is not yet fully understood; however, recent evidence has demonstrated that stem cell exhaustion can lead to premature aging in mice (Vilas et al., 2018). As stem cells age, they lose their self-renewal and regenerative potential, thereby contributing to aging-related tissue dysfunction. Stem cell aging is a complex and multifactorial process, and its underlying mechanisms can be either tissue-specific or common across aging tissues. Common mechanisms of aging include damage to nuclear and mitochondrial DNA, aging-related epigenetic changes, cell cycle alterations, reactive oxygen species (ROS) and mitochondria dysfunction, protein homeostasis disruption, signaling pathway alterations, extrinsic and systematic changes, and dysregulation of autophagy and metabolism (López-Otín et al., 2013) (Figure 2). Stem cell aging is also considered to be one of the most important drivers of organismal aging, which impairs tissue function and regeneration ability, and leads to age-related disorders. Understanding stem cell aging mechanisms will

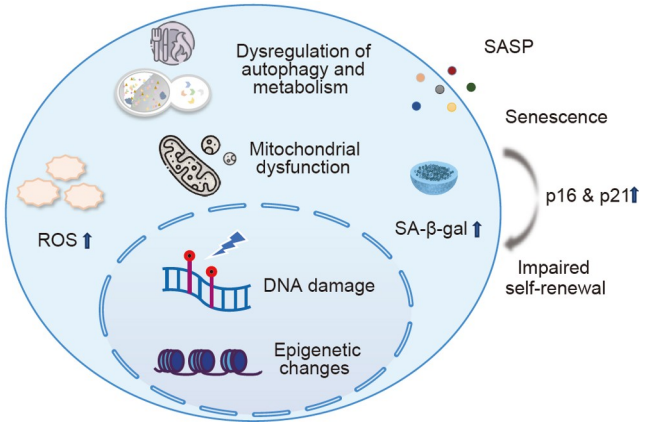

Figure 2 The mechanisms of stem cell aging that lead to impaired regenerative capacity. During aging, stem cells exhibit epigenetic changes, accumulated DNA damage, dysregulation of autophagy and metabolism, as well as senescence and SASP thereby disrupting homeostasis and impairing the regenerative capacity of stem cells. Additionally, senescent markers including elevated senescence-associated SA-β-gal activity, and upregulated Rb-p16 and p53-p21 pathways are induced in aged stem cells.

help provide an avenue to develop new therapeutic interventions to alleviate aging-related diseases, including neurodegenerative diseases (Alzheimer's disease, AD, etc.), sarcopenia and muscular dystrophy (MD), osteoporosis, and lung fibrosis (Wang and Ng, 2022).

Mechanism of stem cell dysfunction during aging

Genetic lesions caused by endogenous or exogenous stress threaten both the survival and function of stem cells, particularly adult stem cells that remain within their niche for an extended period, rendering them prone to accumulate DNA damage and replication-associated mutations (Vitale et al., 2017). Every cell in the human body throughout its life cycle has reportedly experienced hundreds of thousands of genotoxic insults from endogenous metabolic byproducts, such as ROS and inflammation, as well as exogenous agents, including radiation and chemicals in the environment, which can damage DNA (Mani et al., 2020; Yang et al., 2022b). On the other hand, DNA repair ability decreases with age, which may be due to the reduction of ERCC3, PCNA, RPA, XPA, and p53, affecting repair pathways including nucleotide excision repair (NER) (Goukassian et al., 2000). Aged MSCs also show increased DNA damage and importantly, after *in vitro* culture of MSCs, genes associated with base excision repair (BER) and NER, mismatch repair (MMR), and double-strand break repair (DSBR) are all significantly downregulated over time (Galderisi et al., 2009). In addition to shared mechanisms of adult stem cell aging, there are tissue-specific causes and outcomes pertaining to these residential stem cells. For example, hematopoietic stem and progenitor cells (HSPCs) exhibit diminished efficiency in DSBR, which may contribute to genomic instability and continuous accumulation of mutagenic alterations and lead to a hematopoiesis imbalance or even blood and bone marrow cancer. Additionally, the expression levels of DNA damage response (DDR)-related proteins are lower in aged ISCs compared with young ISCs (Watanabe et al., 2019). This genomic damage may impair normal stem cell function, which is detrimental to corresponding tissue homeostasis. Therefore, many types of stem cells, including a large proportion of HSCs and satellite cells, are maintained in a quiescent state. This offers a protective mechanism against acquired replication damage. Although proliferating stem cells are more susceptible to DNA damage, their repair system is more precise than that of stem cells in a quiescent state (Kanaar et al., 2008; Mi et al., 2022; Sousa-Victor et al., 2014).

In addition to genetic alterations, epigenetic modifications, such as heterochromatin stability, are critical for maintaining normal function of tissue stem cells (Le et al., 2021; Zhang et al., 2020c). Heterochromatin loss, including decreases in H3K9me3, HP1α, SUV39H1, and LAP2β levels, is observed in prematurely-aged human MSCs (WRN-deficient MSCs; a cellular model of Werner syndrome) or MSCs from the elderly (Wu et al., 2018; Zhang et al., 2015b). SIRT3 and SIRT7 are NAD^+-dependent deacetylases of the sirtuin family and decrease during human MSC aging, leading to impaired complex formation of nuclear envelope proteins and heterochromatin-associated proteins (Bi et al., 2020; Diao et al., 2021). Apolipoprotein E (APOE), a component of lipoprotein particles, accumulates in senescent stem cells and functions as a heterochromatin destabilizer, thereby driving cellular senescence (Zhao et al., 2022). A recent study reported that aged mouse MSCs exhibit chromatin status alterations including decreased histone acetylation on promoters and enhancers of osteogenic genes, which impair osteogenesis *in vivo* with age (Pouikli et al., 2021).

Additionally, regulation of DNA methylation is required to support the function of several adult stem cells. The landscape of DNA methylation also changes with age and the epigenetic clock, based on a set of CpG methylation sites, can be used to measure biological aging (Bell et al., 2019). DNA methylation age is a promising molecular estimator for biological age and predicts early disease risk, as well as life expectancy and mortality in different tissues (Bell et al., 2019; Horvath and Raj, 2018). Long-term cultured replication-induced senescent human MSCs show similar DNA methylation changes as do MSCs from elderly donors (Bork et al., 2010). Additionally, recent studies on allogeneic hematopoietic stem cell transplantation (HSCT) have shown that DNA methylation age (i.e., the epigenetic clock) of reconstituted blood reflects the age of the donor and is not influenced by the age of the recipient even up to 17 years following HSCT. This result suggests that the epigenetic age of HSCs is a cell-intrinsic characteristic (Søraas et al., 2019). Furthermore, partial epigenetic reprogramming by expressing factors (Oct4, Sox2, Klf4, and cMyc, thus OSKM) re-

duces the epigenetic age of cells without a loss of cell identity (Browder et al., 2022; Olova et al., 2019). These observations indicate that epigenetic alterations are associated with stem cell aging and can be targeted to rejuvenate aged stem cells.

At the cellular level, stem cell senescence is likely an inducer and/or an outcome of stem cell dysfunction with aging (Cai et al., 2020; Turinetto et al., 2016). Cellular senescence is a permanent state of cell cycle arrest induced by various detrimental stimuli, including replication-induced mutations, DNA damage, epigenetic dysregulation, and toxic agents. Senescence is considered as a hallmark of aging due to tissue regeneration impairments and SASP induction. Considering the harmful impact of senescent cells, various strategies have been developed to selectively eliminate senescent cells, including genetic approach, senolytic drugs, chimeric antigen receptor (CAR) T cells, and senolytic vaccination (Amor et al., 2020; Cai et al., 2020; Suda et al., 2021). Aged MSCs exhibit a series of senescent markers, including elevated senescence-associated β-galactosidase (SA-β-gal) activity, upregulated Rb-p16 and p53-p21 pathways, and persistent DNA damage foci, as well as the secretion of growth factors, proteases, and cytokines (Turinetto et al., 2016). The regenerative function of MuSCs, also known as satellite cells, deteriorates with age because these MuSCs switch from reversible quiescence into a senescent state, and the capacity for self-renewal decreases due to the derepression of p16INK4a (also known as *Cdkn2a*) (Sousa-Victor et al., 2014). Additionally, ISC and MuSC functions are impaired by chronic inflammation that occurs with age.

Stem cell aging is also characterized by metabolic changes, including mitochondria-based metabolic regulation and autophagy. Mitochondrial quality and activity also decline, and mitochondrial dysfunction may induce protein aggregation throughout aging (Sun et al., 2016). The transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is a key regulator of oxidative metabolism and mitochondrial biogenesis and activity. PGC-1α expression decreases with age in both human and mouse skeletal stem cells (SSCs), leading to SSC differentiation into adipocytes rather than osteoblasts. Additionally, PGC-1α regulates the number and proliferation of satellite cells in muscle fiber, at least in part, by altering the stem cell niche, such as through modulating the expression of extracellular matrix (ECM) components (Dinulovic et al., 2016). Autophagy is an evolutionarily conserved self-degradative process associated with health and longevity. It plays a critical role in clearing damaged organelles, removing misfolded or aggregated proteins, and eliminating intracellular pathogens. Basal autophagy is important to maintain the quiescent state of stem cells. However, autophagy is impaired in aged satellite cells, leading to the loss of proteostasis and a decline to the regenerative function of these cells, leading to senescence during aging (García-Prat et al., 2016; Pan et al., 2013). Genetic impairment of autophagy in $Atg7^{\Delta Pax7}$ mice induces mitochondrial dysfunction, increases oxidative stress, and disrupts MuSC homeostasis (García-Prat et al., 2016). Additionally, autophagy regulates oxidative metabolism and removes activated mitochondria in HSCs, an essential function that facilitates the establishment or maintenance of HSCs stemness and regenerative potential (Ho et al., 2017). In old HSCs, autophagy loss induces the accumulation of mitochondria and activates the metabolism driving biased myeloid differentiation (Ho et al., 2017). Conversely, disruption of the Beclin 1-BCL2 interaction increases basal autophagic flux and extends both the lifespan and healthspan compared to wild-type littermates (Fernández et al., 2018). Several signal pathways that regulate the metabolism including AMPK, mechanistic target of rapamycin (mTOR), and insulin-IGF signaling also reportedly modulate stem cells. Furthermore, metabolism dysregulation and excessive accumulation of damaging ROS play a role in stem cell aging (Kubben et al., 2016; Schultz and Sinclair, 2016).

Stem cell exhaustion during aging

Adult stem cell exhaustion, defined as the decrease in stem cell number and function, is a hallmark of aging. Stem cell exhaustion is associated with the progressive loss of physiological integrity and the occurrence of multiple age-related pathologies. MSC attrition is observed in multiple progeria-related diseases, including Werner syndrome (WS), Hutchinson-Gilford progeria syndrome (HGPS), Fanconi anemia (FA), and Cockayne syndrome (CS) (Liu et al., 2011; Liu et al., 2014; Ren et al., 2017; Wang et al., 2020d). Decreases in the number and self-renewal ability of bone-marrow MSCs tend to differentiate into adipocytes at the cost of osteoblasts, resulting in both osteoporosis and obesity. HSC exhaustion also contributes to anemia and myelodysplastic syndromes, blood diseases in which stem cells are unable to mature into normal blood cells (Shastri et al., 2017). MuSC exhaustion hinders the regeneration of muscle fibers, which may cause sarcopenia with aging (Blau et al., 2015). Therefore, age-associated stem cell exhaustion hinders the normal function of stem cells and tissues and leads to age-related diseases.

There are many factors leading to stem cell exhaustion that are related to the heterogeneity of stem cells in various tissues. The accumulation of DNA damage in stem cells can induce cellular senescence. The induction of stem cell senescence, such as in MSCs and MuSCs, in turn will cause exhaustion of these cells. Furthermore, epigenetic dysregulation also causes exhaustion. Loss of histone lysine demethylase 4B (KDM4B), a H3K9me3 demethylase, impairs MSCs self-renewal and induces MSCs exhaustion accompanied by increases to senescence-associated hetero-

chromatin foci (SAHF) formation (Deng et al., 2021). Importantly, KDM4B epigenetically removes repressive H3K9me3 and regulates β-catenin/Smad1-mediated transcription, however KDM4B ablation diminishes the stem cell pool and exacerbates skeletal aging and osteoporosis (Deng et al., 2021). Chronic inflammation due to repeated exposure to LPS leads to myeloid differentiation of HSCs, enhances HSCs circulation, and ultimately induces HSCs exhaustion, which mirrors an aging phenotype in mice (Singh et al., 2018). Deletion of Id1, a helix-loop-helix (HLH) protein, facilitates the self-renewal of HSCs in serial bone marrow transplantation (BMT), corresponding to reduced oxidative stress, which protects the HSCs from exhaustion through aging and chronic inflammatory stress (Singh et al., 2018). Furthermore, aged HFSCs inhibit the expression of genes associated with ECM and HFSC-specific cell adhesion. Consequently, diminished cell adhesion and compromised niche lead to the escape of HFSCs, ultimately leading to stem cell exhaustion and tissue degeneration (Zhang et al., 2021b). Adult stem cell exhaustion induced by partial depletion of $Sox2^{+}$ cells triggers cellular senescence and premature tissue aging, including increased kyphosis, hair graying, and reduced fat mass (Vilas et al., 2018). These results suggest that stem cell exhaustion is a critical factor in physiological aging (Vilas et al., 2018). These data partially provide a mechanistic explanation of stem cell exhaustion with aging, and this avenue may be utilized to attenuate stem cell aging.

Summary

Tissue stem cells play an important role in regulating physical functions across a range of organs and systems, which is inherently connected to overall organism health (Oh et al., 2014; Ren et al., 2017). As such, it is critical to understand the mechanisms behind stem cell dysfunction during aging, including DNA damage, epigenetic changes, ROS and mitochondria dysfunction, cellular senescence, and dysregulation of autophagy and metabolism (Figure 2). However, due to the complexity and heterogeneity of stem cells, it remains unclear whether stem cell aging drives organism aging or if it is simply a concomitant phenomenon of organismal aging, and whether aging in one type of stem cell affects the aging of other stem cells. Recent advances in novel stem cell-derived organoid cultures mimic *in vivo* stem cell aging, including the interactions between cells and their microenvironment. Single-cell assays, including single-cell RNA-sequencing (scRNA-seq), and spatial transcriptomics enable us to study aging at the single-cell level, uncover heterogeneous cell populations, and decipher the spatial distribution of aged stem cells. Additionally, recent studies have found that heterochronic parabiosis rejuvenates adult stem cells in aged individuals, especially HSPCs (Ma et al., 2022; Pálovics et al., 2022; Wang et al., 2022a). Taken together, stem cell aging is a central hallmark of aging, and strategies that target stem cell exhaustion during aging hold great promise for developing regenerative medicine and delaying aging and age-associated diseases.

Cellular senescence and the SASP

Aging represents a major risk factor for diverse cancer types, cardiovascular diseases, metabolic disorders, and neurodegenerative pathologies. Although we are yet far from understanding the biological basis of aging, increasing lines of research suggest that targeting the aging process itself can ameliorate many age-related conditions. Senescence is a cellular response characterized by a stable and long-term loss of growth potential and other phenotypic alterations, particularly development of a proinflammatory secretome. Senescence plays essential roles in embryonic development, tissue homeostasis and tumor prevention. However, senescence has also been implicated as a major cause of most age-related diseases. Recent studies have demonstrated that the genetic or pharmacological ablation of senescent cells extends lifespan and improves healthspan. Herein, we review in this section the molecular and pathological links between cellular senescence, the SASP, tissue dysfunction and organ degeneration, and propose novel therapeutic avenues that may lead to improved healthcare in the setting of global aging.

Historic discovery and evolutionary nature of cellular senescence

Cellular senescence, a permanent proliferative arrest, coupled with multiple phenotypic changes, was first reported by Hayflick and Moorhead in early 1960s (Hayflick and Moorhead, 1961). They further noticed that cells derived from malignant tumors do not undergo this form of senescence, a phenomenon implying that senescence may have evolved to prevent cancer development (Hayflick, 1965). Decades later, Sager and colleagues have demonstrated that cellular senescence is a response to potential tumor-inducing stimuli, and formally propose the senescence program as a potent anti-cancer mechanism (Sager et al., 1983; Sager, 1991). Since the 1960s, our insights into cellular senescence and its pathophysiological implications have substantially expanded.

The core of cellular senescence response, is an essentially irreversible arrest of cell cycle progression and cell proliferation. It is now established from multiple lines of evidence that such an arrest constitutes a potent, cell-autonomous anti-cancer mechanism *in vivo* of higher organisms (Campisi, 2013). On the contrary, mammalian species such as humans and mice harboring mutations that prevent or override senescence-associated growth arrest are highly cancer-prone and usually cease at an early age due to

the development of malignant diseases (Wiley and Campisi, 2021).

Pathophysiological implications of senescent cells

Senescent cells typically develop a complex and multicomponent SASP (Acosta et al., 2008; Coppé et al., 2008; Kuilman et al., 2008). In local microenvironments, the SASP acts cell non-autonomously to change the biological behavior of adjacent cells. The SASP is substantially dynamic, heterogeneous, variable and plastic, a feature dependent on the specific cell type and senescence inducer (Basisty et al., 2020a; Hernandez-Segura et al., 2017). Composed of numerous secreted and proinflammatory molecules, the SASP encompasses a wide range of cytokines, chemokines, bioactive lipids, damage-associated molecular patterns (DAMPs, also known as Alarmins) such as high mobility group box 1 (HMGB1), noncoding nucleotides (miRNAs, mtDNA) and even extracellular vesicles (EVs) (Qi et al., 2022; Roger et al., 2021; Song et al., 2020b; Wiley and Campisi, 2021). Chronic inflammation, which is frequently attributable to the SASP development *in vivo*, is a predominant risk factor for diverse age-related pathologies, including but not limited to late-life malignancies (Furman et al., 2017; Furman et al., 2019). Thus, the term "inflammaging" has been employed to refer to chronic inflammation, a typical feature of aged tissues and essentially caused by a long-term accumulation of senescent cells in the course of organismal aging (Cevenini et al., 2013; Franceschi and Campisi, 2014; Furman et al., 2019).

The senescence response can be either beneficial or deleterious, depending on the pathophysiological context. In fact, such a dualism is consistent with the evolutionary theory of antagonistic pleiotropy, which postulates that traits benefit young organisms in their early life stage, can harm the organismal health during the later lifespan (Williams, 1957). In such a case, aging is likely a consequence of the declining force of natural selection in the time course. As one of the beneficial functions, the senescence program protects young organisms from carcinogenetic events. SASP factors can also promote the morphogenesis of certain structures during embryogenesis (Muñoz-Espín et al., 2013; Storer et al., 2013), and initiate parturition in the placenta (Menon et al., 2019). Senescent cells can be observed transiently at sites of tissue damage where they participate in tissue repair, wound healing and regeneration, mainly through secretion of specific SASP factors (Demaria et al., 2014; Ritschka et al., 2017; Sarig et al., 2019).

Senescent cells continue to accumulate with age in various tissues and organs (Bussian et al., 2018; Childs et al., 2018). Whether such an increase is due to increased production or decreased clearance of senescent cells by the immune system, or both, remains an open and intriguing question. Data from transgenic rodent models and pharmacological interventions consistently implicate senescent cells in a large number of age-related pathologies, including multiple cancer types, neurodegenerative disorders, cardiovascular diseases, and various other chronic conditions (Childs et al., 2017; Song et al., 2020a). However, the vast majority of the pathological effects of senescent cells are attributable to development of the SASP, which stands for a rich source of proinflammatory molecules.

Although senescent cells are usually rare, even in aged organs and diseased tissues, single-cell profiling at both the transcriptomic and proteomic levels substantially helps to identify major drivers of cellular senescence during natural aging and in various age-related pathologies. Generally, senescent cells undergo metabolic reprogramming in order to maintain their growth-arrested but viable state and upregulate the expression of genes required to maintain the highly complex, dynamic, and heterogeneous SASP (Basisty et al., 2020a; Wiley and Campisi, 2016). The causes and consequences of these metabolic alterations have been extensively studied with multiple approaches and in different systems (Wiley and Campisi, 2021).

Regulatory mechanisms of the SASP in senescent cells

Although the SASP is a feature typically shared by diverse senescence types, its regulation is considerably complicated, with the composition and pattern subject to influence by the cell type, and the level, duration and attributes of the senescence inducer (Basisty et al., 2020a; Basisty et al., 2020b). Specifically, a time course transcriptomic profiling study has demonstrated that the SASP composition changes in a temporal manner during cellular senescence (Hoare et al., 2016).

The SASP can be regulated at multiple levels including transcription, mRNA stability, translation and extracellular secretion (Malaquin et al., 2016). Further, the SASP development implicates positive autocrine and paracrine feedback loops, underlying a highly sensitive and robust mechanism of global SASP amplification (Acosta et al., 2013; Zhang et al., 2018a). Dynamically regulated at the transcriptional level, the SASP response is subjected to modulation by a range of cytoplasmic and nuclear factors, which generally converge to the CCAAT/enhancer-binding protein β (C/EBPβ) and nuclear factor kappa-B (NF-κB) transcription factors (Acosta et al., 2008; Chien et al., 2011; Kuilman et al., 2008). The SASP program is activated by a sustained DDR signaling, which can be independent of p53, p16 and p21 (the mediators of cell cycle arrest in senescent cells) (Coppé et al., 2008; Rodier et al., 2009). Upon DNA damage events, the ATM kinase phosphorylates the regulatory NF-κB essential modulator (NEMO) in the nucleus, an action that causes NF-κB activation (Miyamoto, 2011; Zhao et al., 2020). During early senescence, the production of interleukin 1α (IL1α), a cell membrane-bound proinflammatory protein, forms a feed-

forward loop to promote C/EBPβ and NF-κB activities and amplify the SASP response (Acosta et al., 2008; Orjalo et al., 2009). Additionally, interaction between IL1α and IL1 receptor (IL1R) maintains the expression of IL6 and IL8, major interleukins generating a positive feedback loop via amplification of C/EBPβ signaling (Kuilman et al., 2008; Orjalo et al., 2009).

The NF-κB transcriptional complex can be activated by p38MAPKα, in a manner independent of the DDR axis (Freund et al., 2011). Alternatively, the transcription factor GATA4 accumulates in senescent cells and induces the upregulation of *IL1α* and *TRAF3IP2* (encodes an E3 ubiquitin ligase), which together activate the NF-κB signaling to initiate and amplify the SASP expression (Kang et al., 2015). Interestingly, GATA4 induces the SASP via a distinct branch of the DDR signaling pathway, which is p53- and p16-independent, to strengthen senescence (Kang et al., 2015). In some cases, the SASP is also regulated by environmental factors, such as oxygenation, the extent of which varies among and within tissues, and governs the expression of SASP-related genes (Coppé et al., 2010; van Vliet et al., 2021). Hypoxia impairs the activity of mTOR, resulting in reduced IL1α translation, decreased NF-κB activity and suppressed SASP induction (Herranz et al., 2015; Laberge et al., 2015). In hypoxic senescent cells, mTOR activity reduction can be mediated by activation of AMPK, a process helping maintain the energy balance upon physiological hypoxia (González et al., 2020; van Vliet et al., 2021). In addition to coding genes, many long non-coding RNAs are recently found to be regulated by NF-κB and a handful of them also feedback regulate NF-κB activity, SASP and cellular senescence (Cai and Han, 2021).

Of note, expression of the SASP is substantially regulated by epigenetic mechanisms, a feature inherently associated with cellular senescence and has increasingly been reported in recent years. Persistent DNA damage signaling causes proteasomal degradation of major histone H3K9 dimethyltransferases, resulting in decreased histone H3 lysine 9 dimethylation, namely H3K9me2, a repressive chromatin modification, especially at promoters of SASP factors, ultimately enhancing IL6 and IL8 production (Takahashi et al., 2012). Enhanced expression of the H3K79 methyltransferase DOT1L during oncogene-induced senescence (OIS) promotes H3K79me2/3 occupancy at IL1α locus, driving a full-spectrum SASP expression (Leon et al., 2021). The upregulation of SASP factors is balanced by a negative feedback loop, whereby the histone variant macroH2A1 activates DDR signaling, which subsequently and conversely removes macroH2A1 from the chromatin of SASP genes, eventually dampening SASP expression (Chen et al., 2015a). The high mobility group box 2 (HMGB2) is a non-histone chromatin-binding protein, which remodels the chromatin architecture and binds to the loci of central SASP genes, preventing their incorporation into transcriptionally repressive heterochromatin regions and facilitating their expression (Aird et al., 2016). More recently, a study revealed that the expression of the histone H3-specific demethylase, namely KDM4, is upregulated during cellular senescence, a process accompanied by diminished H3K9/H3K36 methylation (Zhang et al., 2021a). Indeed, H3K9/H3K36 methylation dynamically changes during senescence, as a partial feature of an unusually permissive chromatin state shaped by KDM4, which is indeed a key SASP modulator during cellular senescence (Zhang et al., 2021a).

In senescent cells, the cytoplasmic DNA acts as a danger signal and can engage innate immune sensing mechanisms to trigger the SASP. Extrachromosomal DNA pieces released into the cytoplasm recruit the cytosolic DNA-sensor cyclic GMP-AMP synthase (cGAS), which catalyzes production of the second messenger cGMP that binds to stimulator of interferon genes (STING) (Chen et al., 2017). As a consequence, both interferon-regulatory factor 3 (IRF3) and NF-κB transcription factor are phosphorylated, stimulating the production of type I interferons and inflammatory cytokines, respectively (Dou et al., 2017b; Glück et al., 2017; Yang et al., 2017). Mechanistically, chromatin protrusions or nuclear budding from the nucleus might represent the major signals to form cytoplasmic chromatin fragments (CCFs), as a consequence of nuclear Lamin B1 loss in senescent cells (Ivanov et al., 2013). CCFs can be recognized by cGAS and engage the cGAS-STING axis, which is critical for the activation of NF-κB complex and the inflammatory SASP (Vizioli et al., 2020). Alternatively, enhanced transcription of long-interspersed element-1 (LINE-1 or L1), a major human retro-transposable element, partly contributes to the accumulation of DNA fragments in cytoplasm of senescent cells (De Cecco et al., 2019). As L1 has a high reverse transcriptase activity, its activation causes cDNA accumulation in the cytoplasm, thus reinforcing cGAS activation to amplify the SASP response in senescent cells (De Cecco et al., 2019; Simon et al., 2019). Different classes of retrotransposable elements including IAP, ERV, LINEs, and SINE B1 are significantly upregulated in the aged mouse brain and kidney, which are among the most sensitive tissues to aging signals (Ghanam et al., 2019). Further, mtDNA fragments derived from dysfunctional mitochondria also contribute to activating the cGAS-STING pathway (Vizioli et al., 2020). Together, accumulation of extranuclear DNA species including CCFs, mtDNA, cDNA, and nuclear buds in senescent cells functionally elicits cGAS-STING signaling to promote full-spectrum SASP expression (Figure 3).

Summary

Cellular senescence is correlated with an essentially irre-

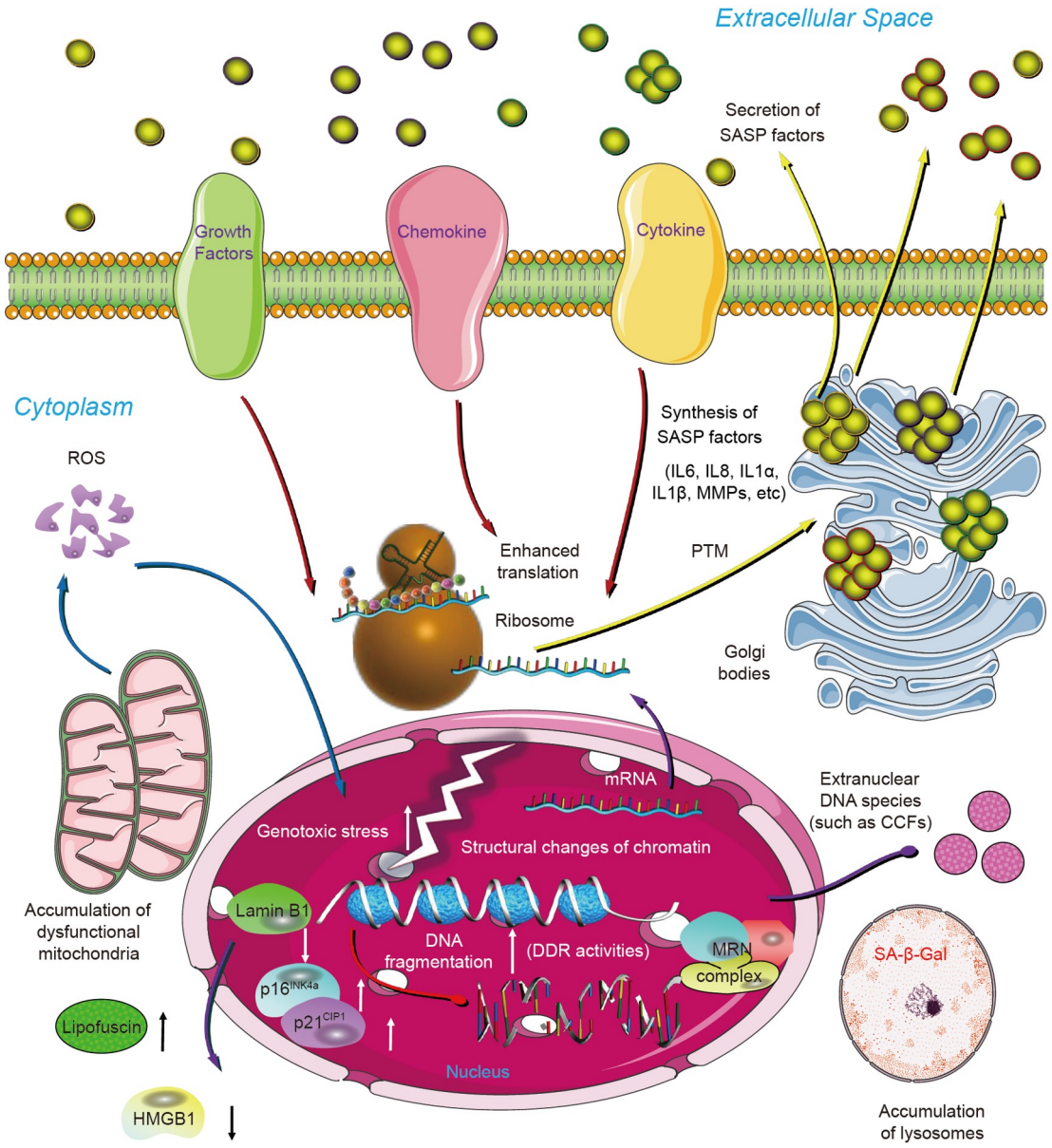

Figure 3 Molecular characteristics of senescent cells. Distinct alterations in molecular pathways of cellular senescence result in morphological changes. The nuclear integrity of senescent cells is compromised due to the loss of Lamin B1 and exclusion of the HMGB1, accompanied by the appearance of extranuclear DNA species, including but not limited to cytoplasmic chromatin fragments (CCFs). Senescent cells have increased lysosomal content, as manifested by elevated SA-β-Gal activity, with an increased number of large but dysfunctional mitochondria that produce high levels of ROS. Biosynthesis and extracellular secretion of a large number of proinflammatory factors, major components of the SASP, is active, robust and chronic, constituting one of the major hallmarks of senescent cells (Song et al., 2020a).

versible growth arrest, apoptosis resistance and frequent acquisition of the chronic, proinflammatory and tissue-destructive SASP. Senescent cells accumulate in various tissues with aging and at sites of pathogenesis in diverse pathologies and degenerative conditions. As a phenomenon of evolutionary pleiotropy, the SASP can contribute to senescence-related inflammation, metabolic dysregulation, stem cell dysfunction, chronic disorders, geriatric syndromes and loss of resilience, all of which are typical aging-related phenotypes and regulated at multiple layers of intercellular signaling network.

Mitochondria and aging

The mitochondrion is a double-membrane-bound organelle found in most eukaryotic cells. As the “powerhouse” of the cell, mitochondria generate the bulk of adenosine triphosphate (ATP), used as a source of chemical energy. In addition to providing cellular energy, mitochondria play an essential role in the biosynthesis of macromolecules, such as lipids, iron-sulfur clusters, and amino acids. The bioenergetic and biosynthetic roles of mitochondria make them function as signaling hubs for diverse biological events in-

cluding apoptosis, innate immune response, and adaptations to stress (Mottis et al., 2019). Mitochondrial function declines during aging (Nunnari and Suomalainen, 2012). Current studies provide evidence that aging interventions such as physical exercise, dietary alterations, and drugs appear to promote healthy aging by mediating the mitochondrial biogenesis, dynamic network, and the quality control pathways (Hood et al., 2019) (Figure 4). Therefore, maintenance of mitochondrial homeostasis has been recognized as a potential pro-longevity mechanism in the aging field.

Mitochondrial biogenesis and dynamic regulation

In aged cells, mitochondria are characterized by the decline in biogenesis, altered morphological network, reduced efficiency of the oxidative phosphorylation (OXPHOS) activity, accumulation of mtDNA mutations, and increased ROS. The impaired mitochondrial function is often observed in tissues with high energy demands, such as the brain, heart, muscles, and liver (Boengler et al., 2017). The decline in mitochondrial biogenesis may result from an age-onset reduction in levels of PPARGC1A, a coactivator of peroxisome proliferator activated receptor gamma (PPARG) that is essential for mitochondrial biogenesis. Overexpression of PPARGC1A in aged muscle led to molecular changes that resemble the patterns observed in skeletal muscle of younger mice. Increasing the activity of PPARGC1A in the muscle cells also leads to resistance to age-related obesity and diabetes and causes a prolonged lifespan with improved brain function in AD mouse models (Boström et al., 2012; Lourenco et al., 2019). Physical exercise, which is the best way to counteract aging, appears to stimulate mitochondrial biogenesis in a wide variety of tissues (Miller et al., 2019).

Mitochondrial morphology undergoes dynamic changes through fusion and fission. This dynamic mitochondrial network is essential to maintain mitochondrial functions and participate in fundamental processes including aging (Liu et al., 2020c). Promoting the function of dynamin-related protein 1 (DRP1), a dynamin-related protein that promotes mitochondrial fission, in midlife prolongs the healthy lifespan of *Drosophila melanogaster* (*Drosophila*). Mitochondrial fusion is essential for diverse longevity pathways in *Caenorhabditis elegans* (*C. elegans*). To summarize, factors involved in mitochondrial biogenesis and dynamics can be viewed as targets to mediate mitochondrial function for lifespan regulation.

Mitochondrial metabolites

Mitochondria can respond to nutritional fluctuation, environmental stresses, or aging of the cells by changing their rates of catabolic and anabolic reactions which lead to the altered production of many metabolites such as ATP, nicotinamide adenine dinucleotide (NAD^+), alpha-ketoglutarate (α-KG), and ROS. Mitochondrial metabolites can serve as substrates, intermediates, or cofactors of metabolic reactions that occur inside and outside of mitochondria. Recent evidence indicates that some of these metabolites can function as secondary messengers whose levels contribute to cellular and organismal aging (Barcena et al., 2018).

NAD^+ is a central metabolic coenzyme involved in cellular energy metabolism and energy production. There is abundant

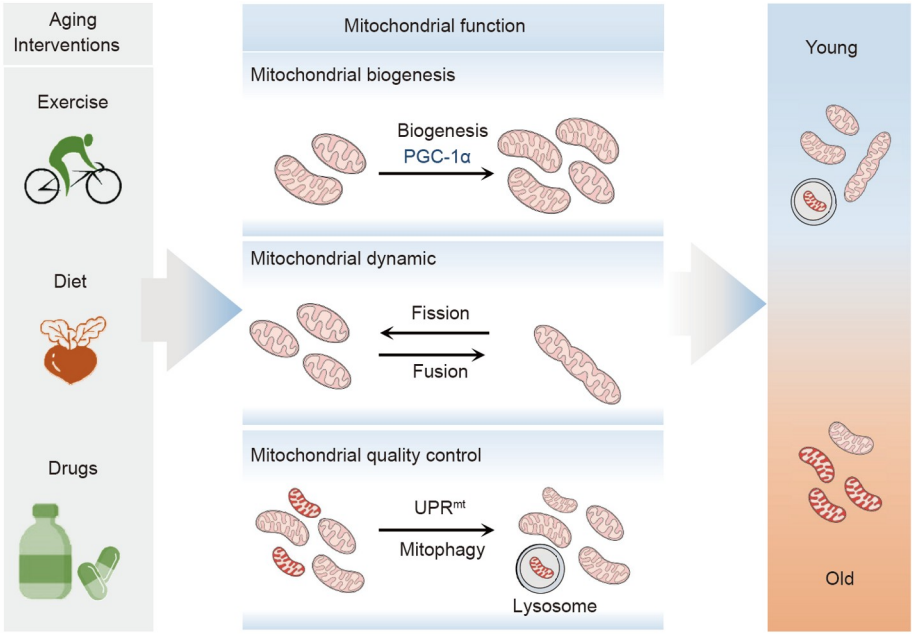

Figure 4 Mitochondrial function and aging. Maintaining mitochondrial homeostasis is essential for delaying aging. Current studies provide evidence that aging interventions such as physical exercise, dietary alterations, and drugs promote longevity through pathways that modify mitochondrial function, including the mitochondrial biogenesis, dynamic network, and mitochondrial quality control pathways.

literature showing that levels of NAD^+ steadily decline with age. Supplementation with different NAD^+ precursors, including nicotinamide riboside (NR) and nicotinamide (NAM) or genetically boosting NAD^+ level through *de novo* NAD^+ synthesis pathway promotes health and longevity in worms, flies, and mice (Katsyuba et al., 2020). α-KG is an intermediary mitochondrial metabolite in the citrate cycle whose levels also naturally decline with age. α-KG is involved in multiple metabolic and cellular pathways, including function as signaling molecules, precursors in the amino acid biosynthesis, and regulators of epigenetic processes. It has been reported that supplementation of α-KG extends lifespan in *C. elegans*, *Drosophila*, and *Mus musculus* (Asadi Shahmirzadi et al., 2020). Thus, adding natural product of mitochondrial metabolites in our daily diet may be a promising way to affect human aging.

Mitochondrial ROS signaling

The mitochondrial free radical theory of aging proposes that the accumulation of oxidative damage caused by mitochondrial ROS is one of the primary causes of aging. Deleting antioxidant enzymes, in general, yields a shorter lifespan in mice. However, there are increasing examples in which the opposite phenotype was observed: increasing ROS levels results in lifespan extension in model organisms (Barcena et al., 2018). Studies in *C. elegans* found that dietary supplementation of antioxidants, N-acetyl cysteine (NAC) and glutathione (GSH), accelerated aging by inhibiting SKN-1-mediated transcription (Gusarov et al., 2021). However, the long-term well-controlled clinical trials showed that popular antioxidant supplements, including NAC, GSH, vitamin E, and vitamin C, fail to show any significant health benefits (Barcena et al., 2018). ROS was initially recognized as unwanted bioproducts that are detrimental to cells. It is now widely accepted that ROS are also signaling molecules essential for various cellular stress response pathways. A recent study provided the Oximouse dataset, a comprehensive and quantitative mapping of the mouse cysteine redox proteome *in vivo*, which can be used as a framework for understanding the mechanism of redox regulation in physiology and aging (Xiao et al., 2020). The relationship between ROS and aging is complex, and this is still an area of much debate. Therefore, accurate detection of ROS targets, including proteins, lipids, and DNA, as well as their roles during aging can help us precisely mediate ROS signaling to delay aging.

Mitochondrial unfolded protein response (UPR^{mt})

Mitochondria are composed of more than a thousand proteins. Only 13 proteins are encoded by the mitochondrial genome (12 in *C. elegans*). The bi-directional communication between mitochondrial and nuclear genomes is extremely important for maintaining a healthy mitochondrial proteome (Mottis et al., 2019). The UPR^{mt} is a mitochondrial-to-nuclear signaling pathway that requires chromatin remodeling and the transcription factor ATFS-1 to induce the expression of the nuclear-encoded mitochondrial chaperones and proteases for reestablishing mitochondrial proteostasis under stress conditions including aging (Bar-Ziv et al., 2020; Nargund et al., 2012; Tian et al., 2016; Zhu et al., 2020).

Inhibition of mitochondrial electronic respiratory chain (ETC) activity extends lifespan in many organisms, and work with *C. elegans* has shown that the timing of the mitochondrial stress-induced longevity is a crucial determinant of longevity. Later, researchers found that activation of the UPR^{mt} is required for the extended longevity upon mitochondrial stress (Bar-Ziv et al., 2020). Not only perturbations of mitochondrial ETC induce the UPR^{mt}, adding bacterial Colanic Acid (CA) to diet also induces the UPR^{mt} and extends the host lifespan in *C. elegans* (Han et al., 2018).

The UPR^{mt} is typically induced in a cell-autonomous manner. However, recent studies discovered that mild mitochondrial stress within the nervous system can be sensed and responded by the distal tissues via neurotransmitter serotonin, neuropeptides, and the Wnt signaling (Bar-Ziv et al., 2020; Zhang et al., 2018b). UPR^{mt} is also required for the healthspan induced by neuronal-specific epigenetic modifications, which participate in mitochondrial gene expression in *C. elegans* (Yuan et al., 2020a). More importantly, the neuronal mitochondrial stress can also communicate to the germ cells, which promotes the maternal inheritance of elevated levels of mtDNA, thereby passing down a "stress memory of the UPR^{mt} activation" to offspring, enabling descendants with a greater tolerance to stress and a longer lifespan (Zhang and Tian, 2022; Zhang et al., 2021f). Thus, the ability of the nervous system to perceive the stress cues and propagate systemic signals to induce metabolic adaptations in peripheral tissues may result in UPR^{mt} activation and increased longevity of the entire animal. The future of inter-tissue mitochondrial stress coordination has great potential to improve our understanding of the systemic regulation of mitochondrial stress response during aging and may lead to therapeutic advances to improve human health via tissue-specific mitochondrial interventions.

Mitophagy

Elimination of damaged mitochondria via a selective form of autophagy, namely mitophagy, is a mitochondrial quality control pathway conserved from yeast to human. Defects in mitophagy are associated with various pathological consequences such as neurodegenerative diseases, cancer, and aging (Onishi et al., 2021). Loss of PINK1 and PARKIN, the mitophagy regulators, displays a range of Parkinson's disease (PD) phenotypes as well as decreased lifespan in *Drosophila* (Imai, 2020). Overexpression of the mitophagy regulator PINK1 and PARKIN promotes longevity in both *C.*

elegans and *Drosophila* (Onishi et al., 2021). Overexpression of Parkin also counteracts age-related sarcopenia and cardiac dysfunction in mice (Gao et al., 2021). Mitophagy deficiency is emerging as a potential driving force of aging, and thus, interventions targeting mitophagy may have therapeutic potential to counteract aging. Pharmacological screens identified that Urolithin A induces mitophagy and prolongs lifespan in *C. elegans*. Similarly, a screen of endogenous metabolites identified myo-inositol can boost mitophagy and lifespan in *C. elegans* through activating PTEN and PINK1 (Shi et al., 2020b). Moreover, Urolithin A also increases muscle function in rodents. Spermidine triggers mitophagy and delays aging in worms, cultured cells, and mice (Onishi et al., 2021). Recently, computational-experimental screening helped the scientists identify two new mitophagy inducers-Kaempferol and Rhapontigenin. The two drugs increased the survival and functionality of glutamatergic and cholinergic neurons, abrogate amyloid-β and tau pathologies, and improve the animals' memory in the *C. elegans* and mouse model (Xie et al., 2022a). Future attempts to identify small molecules and natural products that target the mitophagy pathway will aid therapeutic approaches to age-onset human disorders associated with mitochondrial dysfunction.

Summary

Whether mitochondrial dysfunction is a cause or consequence of aging and incidences of age-associated diseases is still an ongoing debate. A recent study tried to elucidate this question and showed that gradual loss of mitochondrial NDUFS2, a component of OXPHOS complex I, in the dopaminergic neurons of the substantia nigra area, results in progressive, human-like parkinsonism in mice, suggesting that mitochondrial dysfunction might be a driving force of aging and age-related diseases (González-Rodríguez et al., 2021). Single-cell sequencing have shown that mitochondrial antioxidant genes *IDH1* and *NDUFB10* are decreased in aged granulosa cells (GCs) in monkeys. Knocking down these two genes recapitulates the major phenotypes of aged GCs (Wang et al., 2020e). In the future, a better understanding of how mitochondrial function and surveillance pathways affect aging process is of great value for drug development to delay aging.

Metabolism, ER stress and aging

Aging is a process characterized as the progressive decline of physiological functions with loss of metabolic fitness. As a primary risk factor for metabolic disorders such as obesity, diabetes and cardiovascular diseases in mammals, aging is intrinsically linked to the dysfunction of metabolic organs (López-Otín et al., 2013). In both vertebrates and invertebrates, caloric/dietary restriction has been established as the most powerful and successful strategy for aging intervention that restores the function of multiple organs to extend both healthspan and lifespan, underscoring the essential role of nutrient metabolism in health and longevity (Green et al., 2022). A large number of studies using genetic, dietary and pharmacological approaches have uncovered many key aspects in metabolic regulation of aging (Kenyon, 2010; Smith et al., 2020). Here we discuss recent advances in our understanding of the molecular connections between metabolism and aging. We mainly focus on the physiological interplays between nutrient sensing machinery consisting of key components including mTOR, AMPK and Sirtuins, hormonal signaling networks such as the insulin/IGF axis, and stress response pathways like the unfolded protein response (UPR) at the endoplasmic reticulum (ER), which are all implicated in linking metabolic homeostasis to lifespan control (Figure 5). Based on findings from a diversity of model organisms including *C. elegans* (worm), *Drosophila* (fruit fly) as well as rodents, we further outline the conserved ER stress pathway in the systemic modulation of aging and metabolism, which may offer previously unrecognized translational opportunities for promoting metabolic fitness and healthy aging.

Nutrition, metabolism, and longevity

Since the discovery of the impact of restricted food intake upon lifespan in rats, a great number of investigations have demonstrated the beneficial effects of caloric restriction without malnutrition on longevity in multiple model organisms including worms, flies and fishes, as well as in mice and primates with improvement of healthspan (Green et al., 2022; Huang et al., 2022; Ma et al., 2020). Caloric restriction can systemically elicit a broad range of metabolic alterations, which are also found to be responsible for alleviating age-related metabolic disorders. It has been shown to more effectively delay aging than voluntary exercise in mice even in the face of dietary obesity (Green et al., 2017). Moreover, such favorable changes in metabolic pathways represent the profound actions from intracellular nutrient-sensing nodes coupled with regulatory effects of metabolic hormones as well as input from stress signals. For instance, in contrast to starvation that induces a shift of energy source from glucose to lipids through mobilizing fat in the adipose tissue for mitochondrial β-oxidation to produce ketones as energy fuels (Shao et al., 2014), calorie-restricted animals primarily use glucose from food as the fuel to induce fatty acid synthesis in the liver while activating mitochondrial β-oxidation to increase lipid turnover, thereby leading to reductions in circulating and stored lipids including triglycerides, cholesterol and phospholipids after meal (Bruss et al., 2010). Transcriptomics analyses of metabolic tissues also have revealed that caloric restriction not only results in reprogramming of glucose, lipid, and amino acid metabolism, but also evokes

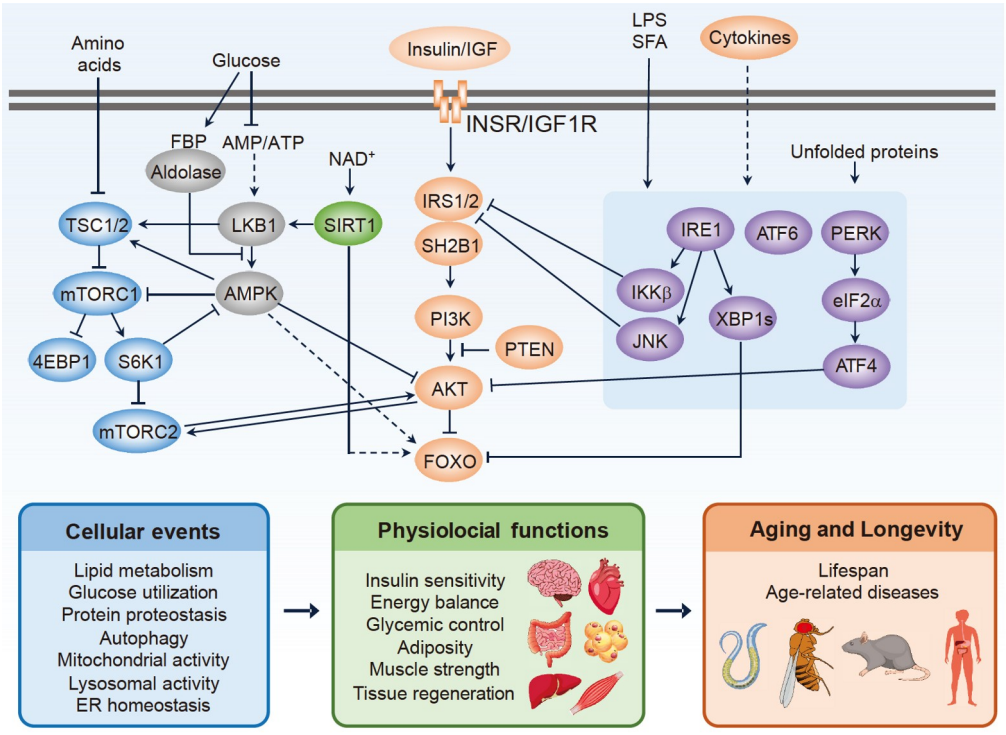

Figure 5 Nutrient-sensing and ER stress response in metabolic control of aging and longevity. Shown are signaling events of key components of nutrient-sensing complexes including mTOR, AMPK and SIRT1 in connection with the insulin/IGF axis as well as the UPR^{ER} pathways in response to a diversity of external stimuli, which have been critically implicated in metabolic adaptation and lifespan regulation. Elucidation of the mechanisms that link nutrition, cell stress response and age-associated metabolic dysfunctions at molecular, cellular and physiological levels will provide novel insights into the molecular basis of aging and offer new intervention strategies for promoting metabolic fitness and healthspan/lifespan in humans.

beneficial changes in RNA dysregulation, cellular senescence, mitochondrial dysfunction, peroxisome import/biogenesis, and metabolic inflammation (Green et al., 2017; Zhou et al., 2012). Intriguingly, recent studies have demonstrated that restricted intake of select nutrients such as protein and specific amino acids, rather than that of total calories alone, as well as meal timing, age of intervention, and even food perception, can exert more prominent impacts on health and aging (Fontana and Partridge, 2015). Therefore, it is conceivable that complex molecular and physiological mechanisms are operative to mediate the profound effects of nutritional inputs, leading to beneficial metabolic alterations in the ultimate control of longevity.

Insulin/IGF signaling and nutrient sensing in lifespan regulation

Hormonal signaling and nutrient sensing are metabolically connected in coordinating their regulatory actions upon health and longevity (Figure 5). The insulin/IGF pathway, an evolutionarily conserved mechanism in regulating nutrient metabolism across species, can be perturbed by caloric restriction (Barbieri et al., 2003). In response to nutrient availability, insulin and IGF1 in mammals, and multiple insulin-like peptides (ILPs) in *Drosophila*, trigger the activation of the conserved PI3K/Akt axis to suppress the transcriptional activity of *FoxO* in metabolic organs (Figure 5), thereby coordinating energy uptake and systemic anabolism (Teleman, 2010). In addition, insulin/IGF signaling can also regulate gene expression programs involved in autophagy, DNA repair, proteolysis, protein translation, mitochondrial homeostasis, and stress resistance as well (Fontana and Partridge, 2015). Genetic studies in model organisms clearly demonstrate the pivotal role of insulin/IGF signaling in the control of longevity (Partridge et al., 2011). For example, ablation of neuroendocrine cells expressing ILP2/3/5 in the brain leads to an increase of median lifespan by ~30% in *Drosophila*. Moreover, flies bearing mutations within the genes encoding the single insulin receptor (*InR*), insulin-receptor substrate (*dIRS*/*chico*), and their adaptor protein (*dSH2B*/*Lnk*) all exhibit >30% longer lifespan than wile-type flies (Partridge et al., 2011; Song et al., 2010). In addition, activation of the single *FoxO*, which is inhibited by phosphorylation via the InR/IRS/Akt axis, in multiple tissues such as the fat body, muscle or brain, all results in a robust lifespan extension in *Drosophila* (Martins et al., 2016). Other components of the insulin/IGF signaling cascade, including Pten, ERK and 4EBP, are also found to regulate the lifespan in flies (Partridge et al., 2011). Similarly, the rejuvenating effects of blocking insulin/IGF signaling are also observed in mammals. Both $Igf1r^{+/-}$ and $Irs1^{-/-}$ mice are documented to be long-lived, and polymorphisms in *IGF1R* and *FOXO3A* are found to be associated with human longevity (Fontana

and Partridge, 2015). Notably, knockout of the *InR* in fat tissue or *Irs2* in the brain has been shown to efficiently extend the lifespan in mice (Taguchi et al., 2007). These findings reveal tissue-specific actions of insulin/IGF signaling in regulating longevity across species.

Numerous studies have also established that in addition to insulin/IGF signaling, multiple nutrient sensors exist to regulate longevity in response to nutritional input and metabolic state in both *Drosophila* and mammals. Initial evidence emerges from the findings that flies bearing null-mutation in *dIRS*/*chico* or *FoxO* genes can still respond to caloric restriction in extending lifespan (Min et al., 2008), indicating the involvement of other nutrient-sensing players. Indeed, several intracellular nutrient-sensing machineries are found to critically underlie the genetic modulation of longevity. AMP-activated protein kinase (AMPK), an ancient energy sensor in multiple organisms, can be activated by intracellular changes in AMP/ADP upon different aging interventions such as caloric restriction and exercise, as well as by sensing the absence of fructose-1,6-bisphosphate (FBP) during glucose deprivation (Trefts and Shaw, 2021; Zhang et al., 2017a). Remarkably, AMPK activation by overexpressing the catalytic α subunit in *C. elegans* significantly promotes longevity through maintaining mitochondrial homeostasis and increasing fatty acid oxidation (Burkewitz et al., 2014). Overexpressing the catalytic α subunit specifically in the brain, gut, fat, or muscle tissues of *Drosophila* mimics the caloric-restriction effects and extends lifespan via induction of autophagy (Burkewitz et al., 2014). Biochemical studies also reveal that AMPK inhibits *Akt* and activates *FoxO* (Burkewitz et al., 2014), indicating its potential link to insulin/IGF signaling in the modulation of longevity in flies. However, the exact relationship between mammalian AMPK activation and aging remains controversial, largely due to the heterogeneity of the AMPK protein complexes in different organs.

Mammalian target of rapamycin (mTOR), another key nutrient sensor existing in two distinct complexes, mTORC1 and mTORC2, can be activated by amino acids and growth hormones at nutrient availability (Albert and Hall, 2015). Inhibition of mTOR signaling, through overexpression of TSC1/2 or the dominant-negative forms of TOR or S6K in the fat body or whole flies, has been shown to extend lifespan in a manner that overlaps with caloric restriction (Partridge et al., 2011). Similarly, systemic inhibition by rapamycin of mTROC1 signaling or genetic knockout of *S6K1*, one of the mTORC1 targets, is also documented to significantly extend longevity in mice (Johnson et al., 2013). Interestingly, rapamycin fails to replicate the beneficial effects of caloric restriction on glucose homeostasis and liver metabolism, indicating the likely existence of metabolically independent mechanisms as well (Miller et al., 2014). Moreover, it has been well established that mTOR signaling modulates the insulin/IGF pathway via multiple downstream effectors, including Akt, IRS, and 4EBP, exerting crucial regulatory effects upon autophagy and lipid metabolism (Yoon, 2017). In fact, through an unbiased systems biology study, all the three pathways described above—IIS, mTOR and AMPK pathways, have been found to form a synergistic circuitry mediating dietary restriction-induced longevity; in *C. elegans*, simultaneous modulating the three pathways can extend lifespan by 200% and completely abolishes the longevity effect of dietary restriction (Hou et al., 2016).

Sirtuins (SIRT1-7), the NAD^+-dependent deacetylases that localize to different cellular compartment to regulate various biological processes, are also critical sensors linking nutrient availability to lifespan control (Chang and Guarente, 2014). Remarkably, whereas *Sirt1*/*dSir2* deficiency abrogates the lifespan-prolonging effect of caloric restriction in *Drosophila*, overexpression of SIRT1 or SIRT4 in either fat body or brain can sufficiently extend lifespan in flies (Lee et al., 2019). Furthermore, mouse SIRT1, localizing in both the nucleus and cytoplasm, has been shown to be essential for lifespan extension by caloric restriction. In addition, brain-specific SIRT1 overexpression delays aging and prolongs longevity in mice with preserved mitochondrial activity. Overexpression of SIRT6, which mostly localizes in the nucleus, also results in lifespan extension in mice, and mitochondria-localized SIRT3 is required for the beneficial metabolic effects associated with caloric restriction (Chang and Guarente, 2014). Interestingly, SIRT proteins have been shown to deacetylate major components of insulin/IGF signaling, including IRS, Akt and FoxO, as well as AMPK, thereby exerting differential regulatory impacts upon mitochondrial activity and metabolic homeostasis (Chang and Guarente, 2014). It warrants further dissecting the intricate molecular and cellular interconnections of these nutrient sensing machineries with various metabolic hormonal signaling nodes, which will open up new avenues for precision nutrition intervention in protecting against metabolic dysfunction to promote healthy aging.

The unfolded protein response of the endoplasmic reticulum (UPR^{ER}) and metabolic stress in aging

Another hallmark of aging is dysregulated proteostasis that causes aberrant accumulation of misfolded or unfolded proteins, including those within the ER (Li et al., 2021d; Martínez et al., 2017). ER stress activates three signaling arms of the UPR^{ER} that are mediated by three ER-resident transmembrane proteins, inositol-requiring enzyme 1 (IRE1), PKR-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6) (Hetz et al., 2020). The UPR^{ER} signaling acts to manage ER stress and maintain cellular homeostasis, mainly through increasing chaperon-associated protein folding, attenuating protein translation, and enhancing ER-associated proteasomal de-

gradation (ERAD) (Yan et al., 2020). Recent studies support crucial roles of the UPR^{ER} in improving the survival of organisms under various stress conditions (Chadwick and Lajoie, 2019). Importantly, increasing lines of evidence have further demonstrated that the ER stress sensors, particularly the IRE1 signaling arm, not only act as nutrient and metabolic stress sensors to regulate various metabolic pathways including the insulin signaling cascade (Huang et al., 2019), but also serve as active regulators of aging-related pathologies and lifespan (Figure 5).

Many studies in model organisms have demonstrated multiple mechanisms by which the IRE1 signaling arm regulates aging and longevity. In *C. elegans*, the IRE1-XBP1 pathway can cross-talk with the insulin/IGF signaling axis in lifespan control and is required for the longevity-promoting action of caloric restriction through enhancing ER hormesis and proteostasis (Matai et al., 2019). IRE1 also mediates the tissue-specific lifespan-extending action of the TOR-HIF-1 axis in a nutrient-dependent manner. Moreover, IRE1 is found to be linked to the activation of the SKN-1/Nrf2 antioxidant response in lifespan control (Taylor and Hetz, 2020), which may also mediate the longevity-promoting effect of vitamin D3 in worms. Intriguingly, neuronal or intestinal IRE1-XBP1 pathway has been shown to extend lifespan through remodeling lipid metabolism, as well as enhancing lysosomal activity in a cell non-autonomous manner (Taylor and Hetz, 2020). Remarkably, in the ISCs of *Drosophila*, the IRE1-XBP1 pathway, in contrast to the PERK signaling branch, is cell-autonomously implicated in improving gut homeostasis and age-associated gut overgrowth to regulate lifespan extension (Wang et al., 2014c). Moreover, activation of the IRE1-XBP1 axis has been shown to mediate the metabolic adaptation upon caloric restriction by promoting lipid synthesis and storage in enterocytes of the midgut, which is essential for extending longevity in flies (Luis et al., 2016). It is worth noting that abrogation of IRE1-Xbp1 signaling in the fat body can increase FoxO stability and the expression of its target genes, including *dATGL*/*Bmm*, leading to elevated lipid mobilization with reduced starvation resistance (Zhao et al., 2021a). It remains to be deciphered, however, whether fat body IRE1-Xbp1 pathway can respond to caloric restriction and contribute to the control of longevity. Recent studies have also extensively shown that the mammalian IRE1α signaling arm of the UPR^{ER} is implicated in the control of insulin sensitivity, energy balance and glucose/lipid metabolism, acting in a tissue- or cell type-selective fashion (Huang et al., 2019). As a sensor of metabolic perturbations stemming from nutrient stress, IRE1α has been documented to promote glucagon-induced hepatic gluconeogenesis, regulate starvation response in the liver, and drive obesity-associated adipose tissue inflammation and insulin resistance (Huang et al., 2019; Mao et al., 2011; Shan et al., 2017; Shao et al., 2014). Given the critical roles of age-associated metabolic alterations in maintaining healthspan and longevity, it will be of immense importance to investigate whether and how IRE1α signaling in specific tissues or cell types is functionally linked to the regulation of systemic aging.

In addition, the UPR^{ER} pathways have also been implicated in aging-associated degenerative conditions or brain aging. For instance, IRE1 has been shown to promote neurodegeneration through autophagy-dependent neuron death in a fly model of Parkinson's disease (Yan et al., 2019a), whereas loss of XBP1 is found to accelerate age-related decline in retinal function and neurodegeneration in mice (McLaughlin et al., 2018). Moreover, genetic disruption of IRE1α in the brain or PERK signaling in the dopaminergic neurons can lead to age-associated cognitive decline and motor dysfunction in mice (Cabral-Miranda et al., 2020). Importantly, intervention strategies targeting ER stress by small molecules have shown promising benefits of improving neuronal plasticity and healthspan (Krukowski et al., 2020).

Perspectives

Metabolic control mechanisms of the aging process are operative at molecular, cellular and organismal levels, involving intricate intercellular and inter-organ communications. An increasing number of novel evolutionarily-conserved aging regulators have been uncovered, primarily circulating proteins acting as key players in intercellular or inter-organ cross-talks. For instance, in *Drosophila*, muscle-secreted proteins, referred as to myokines including IGF-binding proteins (IGFBPs), interleukins, and TGF-β members, may parallel with the muscle functions to mediate organism-wide stress responses and metabolic inter-organ dialogues during aging (Demontis et al., 2014; Ding et al., 2021; Owusu-Ansah et al., 2013; Song et al., 2017b). More intriguingly, in mammals, muscle-secreted Myostatin (MSTN)/GDF8, a TGF-β/Activin family member whose mRNA is subjected to stress regulation by IRE1α in the muscle (He et al., 2021), has been shown to impair metabolic functions of other tissues including fat, liver and heart during aging and lifespan reduction (White and LeBrasseur, 2014). In addition, using joint analyses of metabolomes and transcriptomes across multiple tissues and species during aging and regeneration, uridine, an endogenous metabolite, is identified as an evolutionarily conserved key regulator in promoting multiple tissue regeneration and delaying aging (Liu et al., 2022e). Thus, it is increasingly appreciated that inter-organ communications mediated by diverse secreted proteins, as well as other signaling entities such as EVs or metabolites from the host and/or microbiota, have central roles in systemic regulation of aging and metabolic homeostasis (Fafián-Labora and O'Loghlen, 2020). Given the heterogeneity of cell types in tissue homeostasis and aging, advanced multi-omics technologies including single-cell transcriptomics and me-

tabolomics are needed to obtain new insights into the molecular basis underlying the physiological connections between metabolism, cell stress signaling and longevity. Such knowledge may provide new insights into developing therapeutic strategies via targeting metabolic stress pathways to retard aging and counteract age-associated diseases such as diabetes and sarcopenia, thereby promoting both healthspan and lifespan in humans.

Epigenetics and aging

Epigenetics is defined as heritable and reversible changes in gene expression without alterations to primary DNA sequences, which consist of DNA methylation, chromatin remodeling, histone modifications, histone variants, and noncoding RNAs modulation. Epigenetic mechanisms afford a new understanding of various physiological and pathological processes that cannot be fully explained by genetic factors, such as neurodegenerative diseases, metabolic syndromes, cardiovascular diseases, and cancers (Oh and Petronis, 2021; Stonestrom, 2018). Remarkably, epigenetic changes are characterized as another hallmark of aging, due to their perturbations that contribute to a broad range of aging phenotypes (López-Otín et al., 2013; Zhao and Chen, 2022). Given the reversibility of epigenetic modifications, epigenetic-based therapies have recently emerged as a promising means to delaying aging and aging-related diseases. In this section, we illustrate new, groundbreaking discoveries within the aging epigenome and the potential for providing a theoretical basis for both healthy aging and potential therapeutic targets for aging-related diseases.

Epigenetic changes during aging

Aging is accompanied by epigenetic changes in DNA methylation, histone marks, transcription factor binding, and high-order chromatin organization (Booth and Brunet, 2016). Dysfunctions across layers of the gene regulatory landscape interact reciprocally to propel the aging process. Mounting evidence has shown that the dysregulation of chromatin networks leads to transcriptional aberrance and subsequent cellular senescence, tissue, organ, and organismal aging (López-Otín et al., 2013).

DNA methylation is a dynamic process that is carried out by DNA methyltransferases (DNMTs) and was recently recognized as a biomarker for biological age (Noroozi et al., 2021). "Epigenetic clocks" are based on the methylation levels of certain CpG sites (Horvath et al., 2022) and these DNA methylation-based age estimators help measure the cumulative effects of epigenetic drift on aging across a broad spectrum of human tissues and cell types (Horvath, 2013). Significant changes to histone processes, particularly in histone methylation and acetylation, are also frequently observed in senescent cells. For example, H3K27me3 is lost from many promoters, including those of daf-2/IGF1R in *C. elegans*, suppressing its demethylase UTX-1 and increasing the worm's lifespan in a daf-16/FOXO-dependent manner (Jin et al., 2011). Loss of KDM4B, a histone H3K9 demethylase in MSCs, exacerbates skeletal aging and osteoporosis (Deng et al., 2021). H3K79me2/3 is another histone mark that contributes to the DDR, and its sole methyltransferase DOT1L is recognized as an epigenetic regulator of SASP (Leon et al., 2021).

Several recent studies have revealed the evolutionarily conserved roles of histone acetylation in modulating aging and promoting longevity. For example, it has been described that the epigenetic eraser HDAC4 is polyubiquitylated, degraded in response to all types of senescence, and selectively binds and monitors H3K27ac levels at specific enhancers and super-enhancers that supervise the senescent transcriptome (Di Giorgio et al., 2021). In the mouse or human brain, there is a global reduction of H3K27ac in the gene bodies of aging-upregulated genes, which can be rescued through pharmacological HDAC inhibitor treatment (Cheng et al., 2018). Additionally, histone acetyltransferase KAT7 is required for the quiescence and self-renewal of HSCs, and a CRISPR-based screen identified KAT7 as a driver and interventional target of cellular senescence (Wang et al., 2021d; Yang et al., 2022a). Notably, Sirtuins, a family of NAD^+-dependent deacetylases, are identified as longevity-promoting proteins. For instance, SIRT1 participates in several signaling pathways such as mTOR signaling, NF-κB, FOXO signaling, and AMPK signaling, thereby regulating cellular senescence across species (Chen et al., 2020a). Additionally, SIRT6 deficiency is closely associated with progeroid phenotypes (Korotkov et al., 2021), and SIRT7 safeguards the chromatin architecture to control innate immune signaling during stem cell aging (Bi et al., 2020).

Apart from linear histone post-translational modifications, the large-scale radial repositioning of chromatin structure at each hierarchical layer also converges to significantly progress of aging (Janssen and Lorincz, 2022; Liu et al., 2022b; Liu et al., 2022c). Nuclear deformation, in part due to the diminished core components of nuclear lamina meshworks (i.e., Lamin B1 and LAP2) and heterochromatin proteins (i.e., HP1), is associated with the detachment of periphery heterochromatin (also known as lamina-associated domains [LADs]) from nuclear lamina, resulting in heterochromatin erosion and instability (Deng et al., 2021; Liang et al., 2021). The heterochromatin also becomes globally derepressed and more accessible in senescent cells, demonstrated by the loss of constitutive histone modifications (H3K9me3 and H4K20me3) and increased chromatin accessibility (Zhang et al., 2020c). The relaxed chromatin state segregates from the leakage expression of developmentally restricted genes (e.g., placenta-specific genes [*PSGs*]), as well as repetitive elements that are originally sealed in LADs (e.g., HERV and

LINE1 elements) (De Cecco et al., 2013a; Deng et al., 2019; Liu et al., 2021e; Liu et al., 2022c; Liu et al., 2022d; Peng and Karpen, 2007). Although these elements are typically silenced in young cells, their derepression can trigger an IFN-I response and sterile inflammation, causally linked to cellular senescence, such as in human mesodermal cells (Bi et al., 2020; De Cecco et al., 2019). The derepression of transposable elements is also observed in mouse organs that initiate early aging (Ghanam et al., 2019). Heterochromatin loss during cellular senescence is associated with multiscale three-dimensional genome reorganization and aberrant transcription, including rearranged enhancer-promoter interactions (Luo et al., 2022; Zhang et al., 2021g).

Moreover, long non-coding RNAs, which contribute to various pathophysiological processes, also play a key role in age-related pathways, including DNA damage signaling and telomere maintenance (Aguado et al., 2020; Ghanam et al., 2017). Several lncRNAs cause DNA methylation in the context of senescence and aging, such as *Xist* and *H19* (Grammatikakis et al., 2014). Recent studies have demonstrated that non-coding RNAs derived from pericentromeric repetitive elements impair the DNA binding of CCCTC-binding factor, causing an alteration to chromosomal accessibility and SASP-like inflammatory gene activation (Miyata et al., 2021). Recently, studies have shown that epitranscriptomic regulation, such as N6-methyladenosine (m6A) modification of mRNA, is involved in the regulation of age-related diseases such as AD, a prevalent neurodegeneration disorder (Han et al., 2020; Shafik et al., 2021). Another study on MSCs have determined that m6A mRNA methylation functions in human cellular senescence (Wu et al., 2020b; Wu et al., 2022), in which the methyltransferase METTL3 counteracts premature aging through m6A-dependent stabilization of MIS12 mRNA. Collectively, reorganization mediated by various epigenomic hierarchical layers is a major contributor to aging, identifying potential intervention targets for managing aging and aging-related disorders.

Epigenetic rejuvenation in aging

Considering the reversibility of the epigenetic modifications and chromatin plasticity, several epigenetics-associated interventions have been designed to antagonize aging and age-associated diseases. Pulsed expression of Yamanaka factors partially erases cellular markers of senescent human cells (i.e., decreased H3K9me3 modification) and modestly extends the lifespan of prematurely aged mice (Ocampo et al., 2016). Ectopic expression of *Oct4*, *Sox2*, and *Klf4* in mouse retinal ganglion cells restores youthful DNA methylation patterns and reverses of aging-related vision loss in mice (Lu et al., 2020a). Additionally, knockdown of the H3K27me3 demethylase UTX-1 increases the lifespan of *C. elegans* (Jin et al., 2011; Ni et al., 2012). The yeast protein Sir2 is a histone deacetylase for H4K16 and accounts for silencing, recombination suppression, and extension of the lifespan *in vivo* (Imai et al., 2000). The ablation of epigenetic reader *Baz2b* attenuates age-dependent body-weight gain and prevents cognitive decline in aging mice (Yuan et al., 2020a), whereas BRD4 is required for the SASP and downstream paracrine signaling, critically influencing senescence immune surveillance (Tasdemir et al., 2016). Moreover, the depletion of KAT7 represses $p15^{INK4b}$ transcription via deacetylation of H3K14, and consequently alleviates cellular senescence (Wang et al., 2021d). Interestingly, treatment with a KAT7 inhibitor WM-3835 delays human primary hepatocyte senescence, illustrating the therapeutic potential for the KAT7 inhibitor in clinical settings. Altogether, these findings provide a theoretical basis for exploring possible therapeutic interventions for healthy aging and aging-related diseases.

Summary

Intricate epigenetic changes account for an important aspect of aging progression. The epigenetics-associated interventions illustrated in this section also highlight how epigenetic reprogramming can experimentally shift toward a younger state (Figure 6). Dissecting age-dependent epigenetic changes will help identify the mechanisms underlying aging physiology and pathology, and lead to the development of new interventions using small-molecule drugs and gene therapies to delay or even reverse aging and age-related diseases. Due to the recent development of multi-dimensional and multi-scale omics and a variety of aging models on stem cells and their derivates, it is possible to systematically profile epigenetic alterations at a deeper level throughout aging and expand insights into the underlying biology.

From telomere biology to aging—beyond replicative senescence

Among the aging hallmarks, only the unrelenting telomere shortening is a clear developmental process that acts as a driver of senescence (López-Otín et al., 2013). As the full replication of the terminal DNA cannot be achieved by conventional replication machinery (Gilson and Géli, 2007), telomeres shorten during each cell division. Consequently, telomeric DNA becomes progressively shorter upon each round of somatic replication, ultimately leading to replicative senescence. Therefore, telomere attrition is well known to act as a mitotic clock for cellular senescence. The “clock” starts ticking when telomerase is shut down during mid-embryogenesis in most cells of the human body (Chakravarti et al., 2021a).

In fact, the telomere changes go beyond the developmentally programmed telomere-shortening processes. Mammalian telomeres consist of tens of kilobase tandem repeated sequences, enriched with guanine nucleotides and

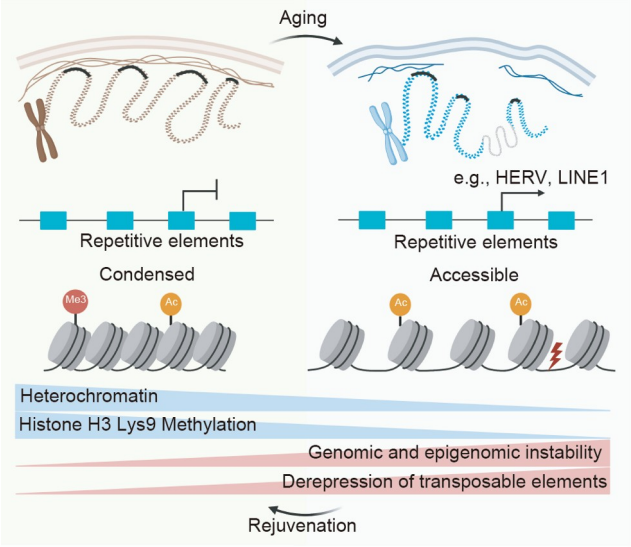

Figure 6 A schematic model of the aging epigenome. This illustration depicts the key epigenetic alterations that occur during aging, including abnormal DNA methylation, altered chromatin modeling, histone modification, and mutations. Simultaneously, a general loss of heterochromatin, derepression of transposable elements, and a varied 3D genome also occur during this process. Given the reversibility of the epigenetic modifications and chromatin plasticity, aging intervention strategies have been proposed based on different layers of epigenetic regulation.

ended in a 50- to 400-nt single-strand 3′ overhang of the chromosome (Figure 7). As telomere DNA is at the chromosome extremities, they are vulnerable. Thus, telomere structure adopts protective chromatin conformations involving telomeric DNA looping (T-loop), specific factors such as the shelterin protein and non-coding RNA (named TERRA). In the field of telomere biology, it is crucial to understand how telomeres couple a hierarchy of proteins ranging from shelterin components to nucleosomal histone modifications to conditionally associating DNA polymerase and the replicative helicases to maintain telomere homeostasis and cellular fate.

Shelterin complex: the gatekeeper for telomere protection

The shelterin complex, is a multi-subunit protein complex bound to the telomeric DNA. Mammalian shelterin comprises six proteins, including the telomeric repeat-binding factor 1 (TERF1), TERF2, ACD shelterin complex subunit and telomerase recruitment factor (TPP1, ACD), protection of telomeres 1 (POT1), TERF1 interacting nuclear factor 2 (TINF2), and TERF2 interacting protein (RAP1, TERF2IP) (Bilaud et al., 1996; Bilaud et al., 1997; Broccoli et al., 1997; de Lange, 2018; Palm and de Lange, 2008). TERF1 and TERF2 share a common TRFH domain and bind to the duplex telomeric repetitive sequence (TTAGGG)*n*. TPP1/POT1 interacts with the single-strand regions at chromosome ends. TINF2 interacts with TERF1, TERF2, and TPP1. RAP1, or Repressor/activator protein 1, is a TERF2-interacting factor. The shelterin complex carries out multiple functions, which are pivotal for telomeres to act against non-programmed cellular senescence via telomere collapse.

One of the most important functions of the shelterin is that it facilitates telomere replication and coordinates telomerase recruitment. Conventional replication machinery encounters two difficulties when it reaches the chromosome ends. One is the end-replication problem that leads to several tens of base pair telomere shortening during each cell division. The other is how telomerase senses and associates with telomeres. Several decades of study show that shelterin is pivotal to solve these two problems. On the one hand, TERF1 recruits the Bloom helicase (BLM) to overcome replication stress (Li et al., 2018a; Zimmermann et al., 2014). On the other hand, the existence of stalled forks requires the recruitment of shelterin at telomeres. TERF2 resolves the topological stress (Ye et al., 2010). TERF1 and TERF2 work separately regarding the replication problems posed by telomeric chromatin: TERF1 prevents the “classical” fork stalling leading to ataxia telangiectasia and Rad3 related (ATR) signaling, while TERF2 removes the topological barriers blocking the progression of the fork posed by telomere replication through nuclease DNA cross-link repair 1B (*DCLRE1B, APOLLO*) (Ye et al., 2010). TERF2 targets the leading telomere with the 5′-exonuclease Apollo, which initiates the 5′ resection (Ye et al., 2010). At the lagging strand, POT1 inhibits Apollo resection (Wu et al., 2012). *POT1* also recruits the single-strand DNA-binding trimeric complex CST (CTC1-STN1-TEN1) to maintain the 5′-strand (Wu et al., 2012). TPP1 regulates the recruitment of telomerase to telomeres and its processivity (Benarroch-Popivker et al., 2016; Chen et al., 2018b; Nandakumar et al., 2012; Xin et al., 2007; Zhong et al., 2012).

Secondly, shelterin maintains higher-order chromatin structure of telomeres and protects telomeres from being recognized by the DNA damage machinery. In addition to the end-replication problem, the stability of telomeric chromatin is difficult to maintain and may result in aberrant recombination or degradation leading to the loss of a large part of the telomeric DNA. This is prevented by specific mechanisms involving the shelterin subunits. The T-loops are key structure to protect chromosome ends. In somatic cells, TERF2 promotes the formation of a T-loop structure and represses non-homologous end joining (NHEJ) and ATM serine/threonine kinase signaling. This architectural solution is efficient to counteract telomere instability, since TERF2 depletion results in T-loop collapse and end-to-end chromosome fusions (Doksani et al., 2013; Griffith et al., 1999; Sarek et al., 2019). During development, TERF2 is dispensable for the T-loops formation, however, POT1 or zinc finger and SCAN domain containing 4 (ZSCAN4) and maybe other factors are required for telomere protection in the absence of TERF2 (Markiewicz-Potoczny et al., 2021;

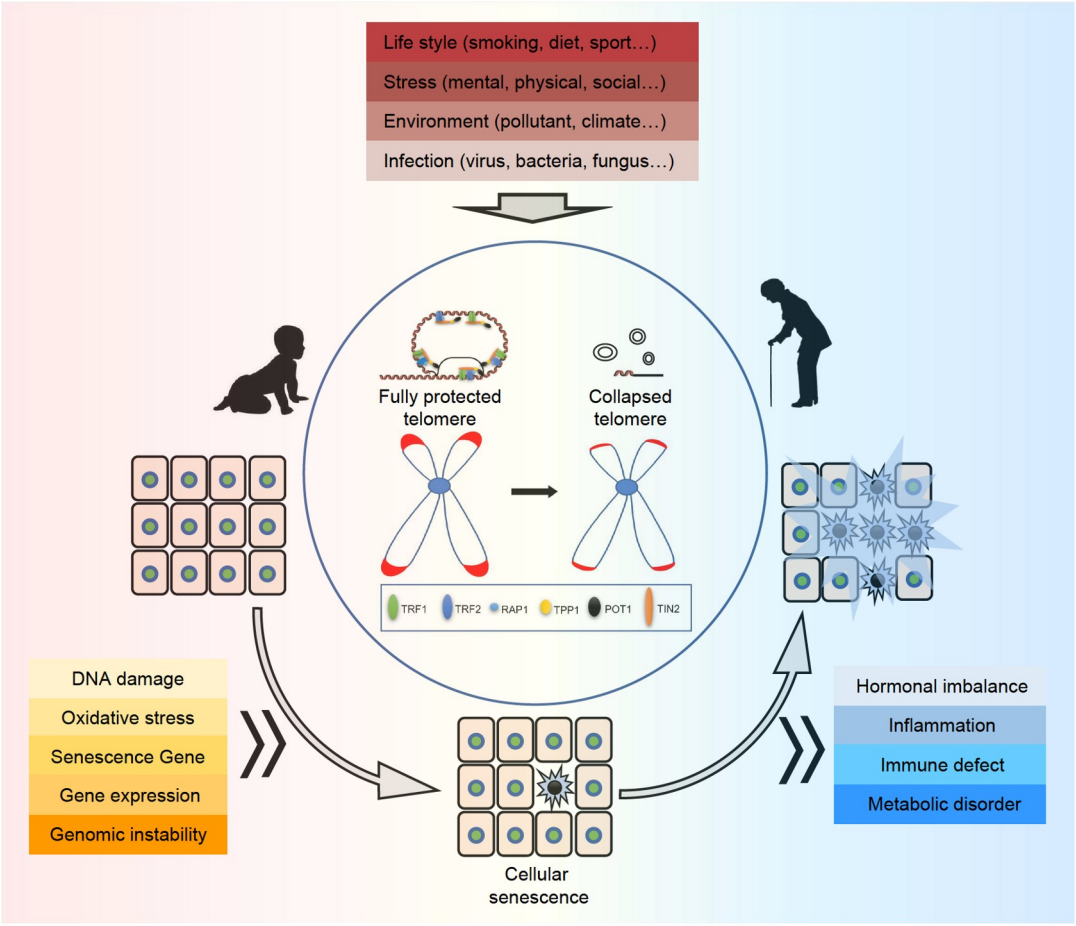

Figure 7 Telomere biology through developmentally programmed aging. Telomeres shorten during each cell division, a developmental process for senescence. Mammalian telomeres are nucleoprotein complexes that are sensitive to four main external factors. Unhealthy life style, stress, environmental change and infection can cause telomere deprotection or structure collapse. Short or unprotected telomeres trigger DNA damage, oxidative stress, senescence, altered gene expression and genomic instability. The accumulation of cells containing collapsed telomere, usually impairs tissue integrity, including hormonal imbalance, inflammation, immune defect and metabolic disorder. This accelerates aging.

Ruis et al., 2021). Together with the data that replication timing regulatory factor 1 (RIF1) is required for the telomere hemostasis in ESCs (Dan et al., 2014), it is clear that there are distinct mechanisms of telomere end protection in pluripotent and somatic cells indicating separate telomere function during tissue development, somatic maintenance and regeneration.

Although the other five shelterin subunits do not seem to be directly involved in T-loop formation and maintenance, they are also crucial for telomere protection. TPP1/POT1 and RAP1 are important repressors of HDR. TERF1 recruits BLM to remove secondary structures formed by the 3′ overhang of telomere and prevent ATR activation at telomeres (Li et al., 2018a; Zimmermann et al., 2014). Removal of the whole shelterin complex from mouse telomeres through conditional deletion of TERF1 and TERF2 confirms that the end-protection problem is specified by six pathways, including ATM and ATR signaling, classical-NHEJ, alt-NHEJ, homologous recombination, and resection (Sfeir and de Lange, 2012).

Recently, several novel specific telomere-associated proteins have been reported to interact with shelterin complex and are involved in the telomere protection. TPP1 recruits CST complex (CTC1-STN1-TEN1), an RPA-like complex that functions as a Polymeraseα/primase accessory factor to act against DSB at 3′ overhang after telomere end resection (Mirman et al., 2018; Wu et al., 2012). TZAP (Telomeric Zinc finger Associated Protein), in competition with TERF1 and TERF2 binds to long telomeres and limit telomere length through resection (Li et al., 2017).

Another function of shelterin in the organization of the telomere structure protection is to regulate heterochromatin replication and homeostasis. TERF2 assists replication fork progression through both telomeres and pericentromeres (Chu et al., 2017; Deng et al., 2009; Lee et al., 2018; Mendez-Bermudez et al., 2018). TERRA, transcribed from mammalian telomere quartets, is involved in the heterochromatin formation and preserves genomic integrity also through TERF2 mediated telomeric R-Loop formation (Feretzaki et al., 2020; Flynn et al., 2011). These findings

reveal unexpected genome-wide concerted actions by telomeres and heterochromatin and invite us to consider the hypothesis that telomeres and heterochromatin share a common functional mechanism for aging and cancer (Mendez-Bermudez et al., 2018; Mendez-Bermudez et al., 2020).

In summary, shelterin dysfunction is detrimental because it causes telomeric and heterochromatic collapses leading to senescence or apoptosis. The dynamics of shelterin complex, together with the choreography of proteins at telomeres during cell cycle, ensures proper maintenance of telomere structure in normal cells. Various cellular and animal models recapitulate the familial and sporadic diseases in human due to the shelterin compromise. All of these diseases share common cellular dysfunctions, including impaired tissue regeneration and proliferative capacity of stem cells, leading to premature telomere shortening (telomeropathies) and developing age-related disorders at a much younger age than the general population (progeroid syndromes). *TERF1* is implicated in stem cell maintenance and proliferation in a mouse model (Karlseder et al., 2003). Progressive repression of TERF1 in aging mice mimics human age-related hematological disorders, developing telomere shortening, bone marrow failure and comparatively short lifespan (Beier et al., 2012). *Terf2* knockout mice are embryo lethal (E13.5d), thus it is mandatory to use conditional mouse models to study its function in different tissues. *Terf2* tissue-specific knockout mice exhibited severe DNA damage, telomere fusion, and chromosome instability, finally leading to tissue specific degeneration (Muñoz et al., 2005). *Terf2* knockout in zebrafish shows an evolutionary trajectory of TERF2 in neurodevelopment and aging (Ying et al., 2022). RAP1 deficient MSCs exhibit enhanced self-renewal, whereas RAP1 deficient hNSCs do not, indicating a lineage specific role for RAP1 in human adult stem cells (Zhang et al., 2019). Mutations in *TINF2* result in earlier clinical symptoms of dyskeratosis congenita (DKC) and organ failure at a younger age, suggesting that TINF2 has particular role in telomerase regulation that is critical for preventing the development of DKC. Overall, the shelterin proteins are potential geroprotective factors that are crucial for tissue development and regeneration, and shelterin dysfunction results in aging-related diseases.

Telomere length as a predictor of human aging

Telomeres are known as particularly hard-to-replicate (hereafter named HTR loci) regions and are therefore prompted to generate chromosome instability in case of replication stress. Therefore, telomeres are also considered as "fragile sites" and represent a particular case of HTR regions since they evolve specific mechanisms to protect them from fragility. Uncompensated fragile telomeres subsequently lead to telomere shortening and the loss of genomic integrity by chromosome rearrangement. All these triggers cellular fate to cell cycle arrest, senescence, apoptosis or malignant transformation.

The dynamics of TL have been studied extensively as a biomarker of human aging and a risk factor for age-related diseases. The TL in most tissues declines with age. The TL in leukocyte or peripheral blood mononuclear cell (PBMC) reflects systemic influences on telomere maintenance across human tissues (Demanelis et al., 2020). An increased risk of incidence and mortality of aging-related diseases (Hampton, 2011), such as diabetes, cardiovascular disease, depression and cognitive decline, cancer and susceptibility to infection with poor immune function (Mender et al., 2020; Tamura et al., 2016) are predicted by and/or associated with short telomeres.

TL is also a biomarker of stress and unhealthy situation of premature aging. TL homeostasis responds highly interactive to life style (Epel and Prather, 2018) and environment change (Garrett-Bakelman et al., 2019), including pathogen infection. For example, COVID-19 virus causes a significant telomere shortening in the infected cohort (Mongelli et al., 2021) (Figure 7).

As very short telomeres are usually inherited in telomere syndrome mutation families and heritability contributes to human TL variation ranging from 30% to 80%, the individual TL variation can explain the link between stress and aging. Thus, the severity of telomere attrition is a biomarker of aging trajectories and a well-known risk of age-related disease. Critically short TL can be early signs to intercept individuals at risk of developing age-related diseases. The degree of telomere shortness can also account for the accumulation of stress exposure during life course. For example, patients within the higher percentiles of short telomeres have higher risk of developing severe COVID-19 pathologies (Sanchez-Vazquez et al., 2021).

Oxidative stress links stressor exposure to telomere attrition

The mechanisms by which environmental change-induced telomere attrition are still elusive (Figure 7). An emerging notion is that telomere dysfunction and oxidative stress are directly coupled phenomena since: (i) oxidative stress shortens telomeres while antioxidants decelerate this process (von Zglinicki, 2002). Individuals submitted to a diet rich in fibers, vitamins and unsaturated fatty acids tend to have longer telomeres and lower oxidative stress. Caffeine consumption, another strong antioxidant agent, has also been correlated with longer leukocytes TL (Liu et al., 2016). (ii) Telomeric DNA is easily oxidized due to its high content in guanine and 8-oxo-guanine disrupts the association of telomere protective factors and telomerase, leading to a high production of DNA breaks and telomere collapse (Fouquerel et al., 2019; Saretzki and von Zglinicki, 2003). (iii) Telomere dysfunction activates p53 which in turn binds and represses *PPARG* coactivator 1 alpha

(*PPARGC1A*) and *PPARGC1B* promoters, thereby leading to mitochondrial dysfunction (Sahin et al., 2011). Therefore, telomeres and mitochondria appear to be part of a positive feedback loop where dysfunction of one triggers dysfunction of the other, and vice versa. Thus a tempting hypothesis is that developmentally-regulated telomere dysfunction initiates such an "explosive" loop driving a proinflammatory and pro-aging environment. Indeed, telomere dysfunction results in inflammatory pathogenesis and environmental stress (Chakravarti et al., 2021b; Wang et al., 2020b). Telomere dysfunction also induces immuno-deficiency through the accumulation of ROS-induced proinflammatory factors (Mender et al., 2020). Moreover, telomere dynamics directly determines the capacity of lymphocytes expansion and telomere binding proteins are also involved in recruiting and activating natural killer (NK) cells (Biroccio et al., 2013).

Stress exposure also elevates glucocorticoid, which seems to modify telomere homeostasis. However, the mechanisms involved remain elusive (Angelier et al., 2018). One possible hypothesis is that glucocorticoid increases oxidative stress, while the others believe that glucocorticoid alters the rearrangement of mitochondrial metabolism. Thus, oxidative stress may be a mechanism that links telomere dysfunction to deteriorated conditions, including high inflammation, immunodeficiency, mitochondrial dysfunction and hormonal imbalance, which favors systemic aging (Figure 7).

Evidence for changes in telomeres during long-lived post-mitotic cells (LLPMCs) aging remains limited. As described above, cellular senescence and aging have been essentially described in replicative cells. However, another type of aging process at the cellular level concerns terminally differentiated LLPMC, such as myofibers and neurons whose function declines with age with no overt sign of cellular senescence (Mattson and Arumugam, 2018; Milde et al., 2015; Vagnoni and Bullock, 2016; Vagnoni and Bullock, 2018). LLPMCs can live for decades in the organism and progressively lose their functional capacities with age without exhibiting any clear sign of senescence. If stem cell division can be considered as a rejuvenation mechanism, the situation is different for LLPMCs, which mainly rely on their intracellular capacities of repair and renewal (e.g., autophagy and mitophagy) (Bua et al., 2006; Fritzen et al., 2016; Kraytsberg et al., 2006; Linnane et al., 1989; Loos et al., 2017; Rubinsztein et al., 2011; Sakuma and Tanaka, 2016; Terman et al., 2010; von Zglinicki et al., 2021). How the cascade of age-related events triggers LLPMC aging is still unclear.

The current view is that telomere dynamics do not play an important role during LLPMC aging since telomere shortening is intrinsically linked to cell division, rather than as a clock for LLPMC aging. However, a few publications report telomere shortening during cell differentiation (Flores et al., 2008), muscle and fat aging (Carneiro et al., 2016; Daniali et al., 2013) and in stressed neurons (Mamdani et al., 2015; von Zglinicki, 2002). However, in these studies, it is impossible to decipher whether the telomere shortening is linked to the division of progenitor cells involved in tissue renewal and stress-response, or whether it is the consequence of a telomere maintenance-mechanism specific to LLPMCs. Addressing the role of telomeres in LLPMC functioning and aging, recent findings have unveiled the existence of TL and shelterin changes during LLPMC aging (Robin et al., 2020; Wagner et al., 2017).

Therefore, the current model for LLPMC aging is the accumulation of molecular damages, telomere dysfunction, mitochondrial dysfunction and oxidative stress that will progressively alter the tissue integrity. However, the precise age-dependent mechanisms leading to a progressive increase in ROS and mitochondrial dysfunction in aging LLPMCs are still unknown. In particular, it is unclear whether this increase is caused by a developmentally regulated clock or simply a cumulative effect.

In summary, a wealth of recent publications indicate that telomeres are structurally and functionally reorganized in aging LLPMCs. Whether these changes contribute to their progressive functional decline warrants further study.

Summary

In conclusion, the fundamental acknowledgment of telomere biology has illuminated mechanisms central to many major aging-related diseases. As a key driver of aging, incomplete replication of telomeres controls the balance between tissue renewal and energy consumption. In this context, how telomere dysfunction is transformed into genome-wide signals and promotes tissue homeostasis remains to be unveiled. The dynamic interaction between telomere maintenance, energy consumption, and metabolism, and maybe also other hallmarks of aging can develop a new therapeutic strategy to eliminate senescent cells and prevent individual aging at the early stage.

Age-associated alterations in genome stability: mechanisms and interventions

Genomic instability is a hallmark of aging (López-Otín et al., 2013). Accumulating evidence has delineated that age contributes to the impairment of the guarantee mechanisms of genome stability, and the decline of genome stability can also inversely accelerate the process of aging. Genomic instability is characterized by multiple features, including the occurrence and accumulation of DNA damage, activation of retrotransposons, telomere attrition, and replicative stress. Here, due to the page limit, we only focus on the former two aspects.

DNA damage, DNA repair and aging

DNA damage, one of the main factors contributing to genomic instability, can be resolved by different DNA repair pathways, including DSBR (which can be further categorized into homologous recombination (HR), canonical and alternative non-homologous end joining (c-NHEJ and alt-NHEJ)), single-strand break repair (SSBR), BER, NER, MMR, inter-strand crosslink repair (ICL repair, also known as FA pathway), according to the type of DNA lesions (Hoeijmakers, 2009; Schumacher et al., 2021).

DNA damage/repair and aging have a mutual influence. However, the cause-consequence relation between DNA damage/repair and aging is still an intriguing and puzzling scientific question to be explored. Firstly, a large body of evidence has demonstrated that the capacities of multiple DNA repair pathways decline with age, although there is an obvious cell or tissue-type specificity, which requires further study. Mechanistically, reduced expression or inefficient recruitment of critical DNA repair factors and repair-associated regulatory proteins, such as ATM, KU70, XRCC4, LIG4, MRE11, RAD51, BLM, XRCC1 and SIRT6, have been shown to be the possible causes of individual aging, providing valuable hints for the future pharmacological interventions (Ju et al., 2006; Li et al., 2016c; Xu et al., 2015). More interestingly, change of DNA repair also exhibits sex-specific differences. Age-associated difference of DNA damage and mutations between sexes have been observed in multiple species, including human, mouse, rat and fly (Fischer and Riddle, 2018). A recent paper employs peripheral blood lymphocytes to demonstrate that NHEJ capacity is inversely regulated in men and women, and HR and alt-NHEJ are also altered in a sex-dependent fashion (Rall-Scharpf et al., 2021). Whether the sex-related differences in DNA repair and genome stability underlie the different incidence of human diseases between sexes has been neglected for a long time and obviously requires in-depth investigations.

Secondly, impaired DNA repair and increased DNA damage contribute to the onset of aging (Figure 8). Unrepaired DNA damage activates the DDR and the subsequent p53 signaling, and eventually induces cellular senescence and apoptosis, which are both important drivers of individual aging. It is worth noting that senolytics, drugs that specifically eliminate senescent cells, show great potential in extending both the healthspan and lifespan in mice (Martel et al., 2020). Senescent cells secrete large amounts of proinflammatory factors, namely, SASP, and DNA damage can also directly trigger the secretion of inflammatory factors, both contributing to sterile inflammation and inflammaging (Rodier et al., 2009; Yu et al., 2015). Chronic inflammation has been considered to be one of the pillars of age-related diseases (Kennedy et al., 2014). Indeed, a large number of studies have linked DNA damage-associated inflammatory response with human diseases (Chakravarti et al., 2021b; Karakasilioti et al., 2013; McNairn et al., 2019; Tumurkhuu et al., 2016; Yan et al., 2013a), many of which are geriatric diseases. Supporting this idea, senomorphics, another category of drugs, have been found to delay age-related pathologies by blocking SASP (Martel et al., 2020). Moreover, several DNA repair factors also regulate other biological processes that exhibit age-related changes. For instance, PARP1 has been found to detect DNA damage in brain to promote sleep, and sleep can increase DNA repair to maintain cellular homeostasis (Zada et al., 2021). Is PARP1 activity altered during the process of aging due to the decreased amount of its substrate NAD^+ (Gomes et al., 2013)? Does decreased PARP1 activity lead to sleep disturbances, which are associated with neurodegenerative diseases? Future comprehensive studies are needed for addressing these issues to promote our understanding the multi-faced roles of repair factors in aging regulation.

Alterations in DNA repair capacity are closely related to the phenotype of premature aging and longevity. Deficiencies in DDR genes or genes involved in DNA repair pathways such as HR and NER have been linked with human progeria syndromes, including WS, Ataxia telangiectasia, Seckel syndrome, Bloom syndrome, CS and Xeroderma pigmentosum (Chen et al., 2020c; Liu et al., 2011; Zhang et al., 2015b). The rare but well-known HGPS is also represented by deficiencies in multiple DNA repair pathways (Gonzalo and Kreienkamp, 2015). Although the classical function of the pathogenic genes has been well characterized, their novel roles in regulating genome stability are still to be investigated. A recent paper has shown that Werner protein (WRN) participates in the regulation of HR/NHEJ pathway choice in a CDK2-dependent manner (Lee et al., 2021). Moreover, in HGPS cells, the NER factor XPA mysteriously colocalizes with γ-H2AX, the marker of DSB (Liu et al., 2008), and the BER capacity also decreases, attributing to the reduced expression or activity of several BER factors in HGPS cells (Maynard et al., 2019). Elucidating the novel roles of pathogenic, repair-associated genes and discovering the regulatory mechanisms of DNA repair in premature diseases will help us develop strategies to treat these diseases, and may also provide hints for developing ways to delay the onset of physiological aging in the future.

DNA repair and longevity: lessons from non-classical animal models

Comparative biology provides us a unique viewpoint to understand the relationship between DNA repair and longevity. Long-lived species undergo a longer period of life, therefore, theoretically, investing in a robust DNA repair system can more efficiently maintain their genomes stable. Thus, it is believed that DNA repair capacity is stronger in species with a longer lifespan. A recent study assembles the

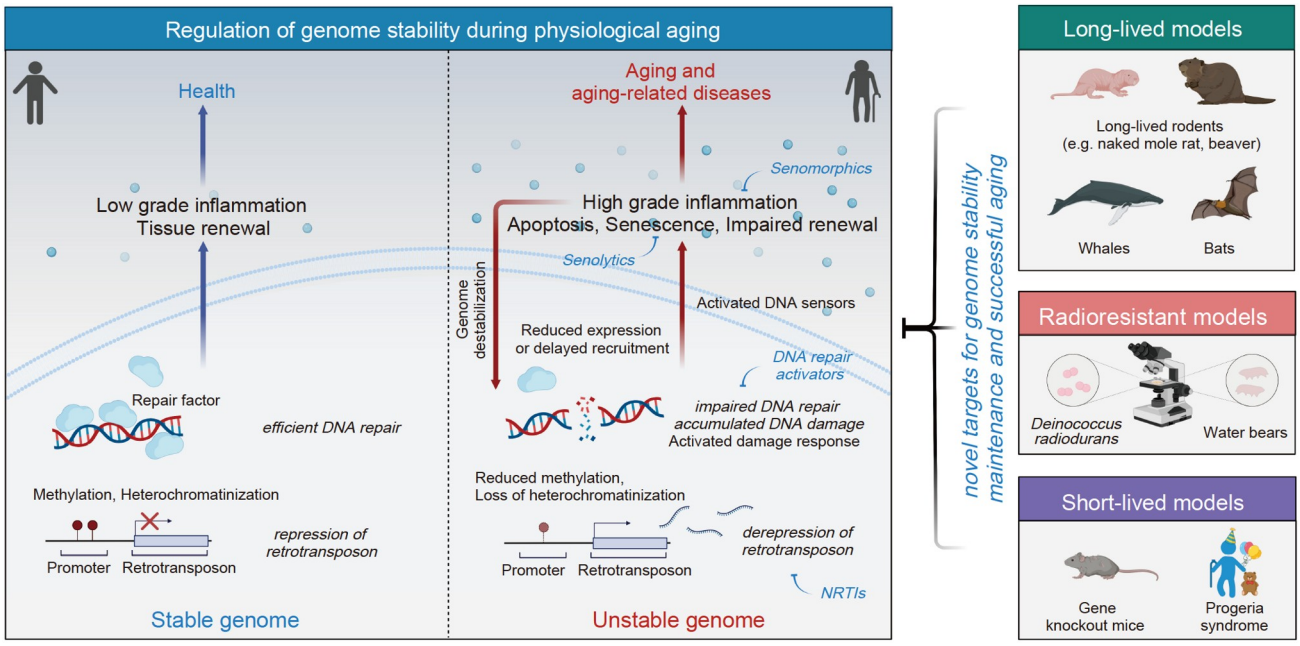

Figure 8 Regulation of genome stability and aging. The genome is maintained stable in young individuals. Damaged DNA can be timely repaired and the transcription of retrotransposons are repressed via heterochromatinization of promoters by multiple mechanisms such as DNA methylation. With increasing age, reduced expression or delayed recruitment of repair factors, and the loss of heterochromatinization in the promoter regions of retrotransposons, both contribute to genomic instability, activating the checkpoint and leading to apoptosis, senescence and dysfunction in renewal. In addition, the DNA damage per se can induce inflammatory factor secretion, and the cytosolic DNA fragments derived from DNA damage and retrotransposons can activate DNA sensors to trigger innate immune signaling, inducing high-grade inflammation, and further influence the cell fate of nearby cells, eventually accelerate the process of aging. Several chemicals, which target genome stability-related, aging-promoting steps and which also delay the onset of aging, are illustrated. Moreover, uncovering the regulatory mechanisms of genome stability in long-lived, radioresistant, and short-lived models will provide us novel targets for aging intervention in the future.

genomes of 88 rockfish species, and demonstrates that DNA repair-associated genes are positively selected in long-lived, but not short-lived rockfish species, implying the fundamental role of DNA repair in regulation of lifespan (Kolora et al., 2021). Using a 5-bromodeoxyuridine photolysis assay, a well-known study has shown that the number of UV-induced DNA excision repair sites but not the size of repaired regions positively correlates with the maximum lifespan of 21 mammalian species (Francis et al., 1981). Consistent with this, a similar correlation has also been reported in the primary fibroblasts and non-stimulated lymphocytes of seven primate species (Hall et al., 1984). Intriguingly, using a fluorescence plasmid reactivation assay, a recent study has demonstrated that the efficiency of NER correlates with the environmental sunlight exposure rather than the maximum lifespan, in 18 rodents, while the capacity of DSB repair coevolves with the maximum lifespan (Tian et al., 2019). These controversial conclusions may attribute to the influence of cell types, the phylogeny of species included in the studies, and the methods used for analysis. Moreover, the activity of nuclear apurinic/apyrimidinic (AP) endonuclease (APE) and polymerase β (polβ), both are critical BER factors, does not correlate with the maximum lifespan in the liver and brain of 15 vertebrate endotherm species (Page and Stuart, 2012). Interestingly, another study has reported that the mitochondrial BER positively correlates with the maximum lifespan in liver and heart of mammals, reflected by the activities of mtDNA glycosylase and APE1 (Gredilla et al., 2020). A possible explanation for the difference between nuclear and mitochondrial BER may be that the amount of ROS released from mitochondria is lower in long-lived species (Ku et al., 1993), therefore there is no need to maintain a more efficient BER in the nucleus since the resource is limited.

For a long time, classical animal models with short life cycle and lifespan, such as yeast, worm, fruit fly, mouse and rat, have been employed for studying the mechanism of biology of aging and greatly advanced our knowledge on biology of aging. Recently, dissecting the molecular mechanisms of longevity using long-lived animals as models also received great attention in the field of aging (Figure 8). Studies using long-lived rodents, such as naked mole rat, blind mole rat and beaver, have unveiled unique features of genome stability regulation in these animals (Zhou et al., 2020), which exhibit great promise for clinic translation. For instance, as one of the members of the Sirtuin longevity gene family, SIRT6, which plays key roles in regulating multiple pathways of DNA repair, has an enhanced DNA repair capacity in beaver, compared to the short-lived mouse, and five amino acids within SIRT6 have been proven to be re-

sponsible for the enhancement (Tian et al., 2019). The NER and BER capacity are also upregulated in naked mole rats, compared to mice (Evdokimov et al., 2018). Several bat species have evolved extreme longevity compared to their similar-sized mammals. A list of DNA repair genes including *ATM*, *PRKDC*, *KU80*, *BRCA2* and *RAD50* have been found to be positively selected in bats (Zhang et al., 2013b). Moreover, the expression level of ATP-binding cassette (ABC) transporter ABCB1 is higher in several species of bats than that in human, mediating efficient drug efflux upon genotoxic compound treatment to maintain genome stability (Koh et al., 2019). In addition, the capacity of DNA repair has been demonstrated to be elevated in gray whale, one of the longest-lived mammals (Toren et al., 2020), further indicating the tight link between DNA repair activity and lifespan. Several prokaryotes and invertebrates also provide clues about the resistant mechanisms to radiation, an important source of DSBs. *Deinococcus radiodurans* is extraordinarily resistant to ionizing irradiation (Cox and Battista, 2005), and some unusual adaptations in DNA repair pathways, including the employment of extended synthesis-dependent single-strand DNA annealing and the RecFOR pathway, have been uncovered in *Deinococcus radiodurans* (Cox et al., 2010). However, the mechanisms underlying their robust ability to repair DSBs remain largely unknown. Tardigrades, also known as water bears, is a kind of aquatic invertebrates which exhibit tolerance to extreme environment, including high doses of ionizing irradiation and UV radiation (Horikawa et al., 2013). Different Tardigrade lineages have evolved specific mechanisms to repair DNA damage. Several Tardigrades have additional copies of genes participating in DNA repair, such as *POLE*, *FEN1* and *PARP1* (Kamilari et al., 2019). Surprisingly, several critical HR genes, including *BRCA1*, *NBS1* and *RBBP8* (also known as *CtIP*) are absent in multiple species of Tardigrade, indicating there may be an alternative pathway evolved to repair DSBs in these species (Kamilari et al., 2019). Dissecting the regulatory mechanisms of genome stability in these radioresistant models may provide us clues to develop novel methods to delay the onset of aging.

Transposable elements, genomic instability and aging

In addition to DNA damage and repair, transposable elements are also critical to the maintenance of genome integrity, and accumulating evidence indicates that they participate in the regulation of the process of aging (Figure 8). Approximately 45% of the genome is composed of transposable elements, including long terminal repeat (LTR) retrotransposable elements, long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs). LINE-1 derived sequence accounts for ~17% of the human genome (Richardson et al., 2015), and LINE-1 is a major autonomously active retrotransposon in human, making it a great threat to genome stability. Methylation of LINE-1 5′UTR has been unveiled to contribute to the repression of LINE-1 retrotransposition. In *Dnmt3L*-deficient germ cells, the transcription of multiple retrotransposons, including LINE-1, become activated, implying that DNMT3L-mediated DNA methylation plays an important role in LINE-1 repression (Bourc'his and Bestor, 2004). A recent study has reported that the methylation level of active LINE-1 decreases with age in skeletal muscle, leading to the derepression of LINE-1 (Min et al., 2019). Consistent with this, the RNA expression of multiple retrotransposons, such as LINE-1, SINEs and MusD/ETn, is elevated in liver and muscle during normal aging process (De Cecco et al., 2013b). Consistently, LINE1 and ERV transcript levels are negatively associated with lifespan in mice across different dietary and exercise regiments (Green et al., 2017). Moreover, LINE1 mRNA level is elevated in senescent cells, the liver and adipose tissue of aged mice and also in multiple tissues of *Sirt6* knock-out mice, triggering interferon response and contributing to aging-associated phenotypes (De Cecco et al., 2019; Deng et al., 2019; Liang et al., 2022; Liang et al., 2021; Simon et al., 2019). Deficiency in SIRT7, another member of Sirtuins, can also contribute to derepression of LINE-1, activation of innate immune signaling and mesenchymal stem cell senescence (Bi et al., 2020). Intriguingly, LINE-1 methylation is positively associated with healthy lifestyles, which are influenced by daily intakes of calories, sports and smoking, while adiposity fat mass is negatively associated with LINE-1 methylation, indicating the complex epigenetic regulation of LINE-1 expression (Marques-Rocha et al., 2016). DNMT1 also plays an important role in silencing LINE-1 (Ren et al., 2021). In human neural progenitor cells, disruption of DNMT1 leads to the activation of evolutionarily young, hominoid-specific LINE-1 elements (Jönsson et al., 2019). Consistent with this, the expression of DNA methyltransferase DNMT1 declines during the process of replicative senescence (Young et al., 2003), which may be one important mechanism underlying LINE-1 derepression. An interesting study has uncovered the role of DNMT1-mediated silencing of LINE-1 in the cancer resistance phenotype of the blind mole rat, a long-lived rodent. Mechanistically, the blind mole rat cells only express very low level of DNMT1. Once hyperplasia occurs, decreased DNA methylation will contribute to the activation of LINE-1, further initiating the innate immune response and the subsequent concerted cell death to suppress tumorigenesis (Zhao et al., 2021b). This study indicates that apart from its role in cellular senescence and individual aging, retrotransposons may also function as an anti-tumor mechanism during the process of aging. Normally retrotransposons are located in the heterochromatin region and are maintained in a transcriptionally inactive state. Methyl-CpG-binding protein 2 (MECP2), a protein involved in heterochromatinization,

binds to the 5′UTR of LINE-1 element to inhibit its activity in neurons (Muotri et al., 2010). It is worth noting that the distribution of heterochromatin is altered during cellular senescence, and the decline mainly occurs in the region of repetitive sequences, possibly due to the change of DNA methylation pattern (Sedivy et al., 2008). Several researches have also demonstrated that the chromatin architecture undergoes dramatic change in senescent cells, leading to the relaxation of heterochromatic region and activation of retrotransposons such as LINE-1 (De Cecco et al., 2013a; Deng et al., 2019; Hu et al., 2020; Liang et al., 2022; Ren et al., 2019).

It is well-known that LINE-1 can create DSB, contributing to genomic instability (Gasior et al., 2006). Notably, genotoxic stress can also modulate the activity of transposable elements. Etoposide, the topoisomerase II inhibitor, can induce the retrotransposition of human Alu elements (Hagan et al., 2003). Besides, LINE-1 retrotransposition is also elevated in *ATM*-deficient human NSCs (Coufal et al., 2011). In addition, upon DNA damage or during the aging process, SIRT1, an important regulator of DNA repair, redistributes from repetitive DNA to DSB site, and therefore facilitates the transcriptional activation of repetitive DNA (Oberdoerffer et al., 2008). Similarly, SIRT6 can bind to the 5'UTR of LINE-1, mono-ADP ribosylate KAP-1, and facilitate the package of LINE-1 elements into a transcriptionally repressive state. However, with increasing age, re-localization of SIRT6, possibly towards the site with DNA damage, activates LINE-1 transcription (Van Meter et al., 2014). Notably, a recent study suggests that the BRCA1 and BRCA1-mediated HR process can restrict L1 retrotransposition at multiple steps (Mita et al., 2020). These studies further imply that there is a crosstalk between the regulation of DNA repair and retrotransposition, and the trade-off between the two pathways is a fascinating point to be explored.

Retrotransposons may lead to age-related diseases through a variety of mechanisms. First, the transposition event may cause mutations including insertions, deletions and inversions, further genetically influencing the expression of nearby genes (Gilbert et al., 2002; Han et al., 2005; Taniguchi-Ikeda et al., 2011; Vogt et al., 2014). Second, LINE-1 insertion has been shown to influence gene expression epigenetically. The alteration of the methylation level of LINE-1 promoter not only influences the expression of LINE-1 *per se*, but can also modulate the expression of nearby genes, by which it can function as an alternative promoter or transcribe regulatory noncoding RNAs (Faulkner et al., 2009; Wolff et al., 2010). Moreover, LINE-1 transposition activates the inflammatory response, which is the basis of a number of aging-related diseases. Deficiency of the exonuclease TREX1 increases the cytosolic LINE-1 ssDNA, eventually leading to the neurotoxicity via upregulation of type 1 IFN secretion (Thomas et al., 2017). Last but not the least, LINE-1 activation has also been linked to somatic mosaicism and further influences the neurobiological processes, which may contribute to the onset of human diseases (Baillie et al., 2011; Muotri et al., 2005).

Can restoration of genome stability delay the onset of aging?

Whether improvement of genome stability contributes to a longer lifespan or healthspan? Although DNA repair is considered as the safeguard of genome stability, deficiencies in DNA repair also shorten the lifespan, and reduced DNA damage or DNA repair improvement has been observed in many established strategies for delaying the onset of aging (Ocampo et al., 2016; Sinha et al., 2014), there is no successful attempt to extend lifespan by activating DNA repair pathways. Actually, direct overexpression of DNA repair factors may bring deleterious consequences (Becker et al., 2018; Klein, 2008; Shaposhnikov et al., 2015). Therefore, whether boosting DNA repair in a dose, time point controllable and tissue specific way will extend lifespan is a meaningful subject to be explored. Retrotransposons, such as LINE-1 is another source of genomic instability. Nucleoside reverse transcriptase inhibitors (NRTIs) have been found to inhibit LINE-1 retrotransposon (Dai et al., 2011b). Overexpression of several factors regulating heterochromatin formation, including *Sir2*, *Dicer2* and *Su(var)3-9*, can prevent age-related activation of transposons, and also contributes to lifespan extension in fruit fly (Wood et al., 2016). Two recent exciting studies have shown that inhibition of LINE-1 with NRTI contributes to relieved age-related inflammation, reduced apoptosis in murine tissues and improved healthspan and lifespan of *SIRT6* knockout mice (De Cecco et al., 2019; Simon et al., 2019), indicating that repression of retrotransposons may be beneficial for longevity. Nonetheless, LINE-1 expression is highly elevated during early embryo development, and the kinetics of LINE-1 expression is important for preimplantation development through regulation of chromatin accessibility (Jachowicz et al., 2017). Does LINE-1 have other potential positive roles for individual growth and development (especially the role in regulating the normal function of reproductive system)? Answering this question will promote the application of LINE-1 inhibition agents, such as NRTIs in aging intervention.

Summary

As we have reviewed here, alteration in genome stability is a core biological event with increasing age. Both accumulated DNA damage arising from impaired DNA repair and the derepression of transposons destabilize the genome, and thereby accelerate the onset of aging and aging-related diseases. Exploring the druggable targets involved in the reg-

ulation of genome stability, with the aids of short- or long-lived models, will be a critically important subject to achieve the goal of successful healthy aging in the future.

Cellular senescence bridges aging and cancer

Cancer and aging seem to be two opposite pathophysiological and biological processes. Cancer is viewed as uncontrolled proliferation, whereas senescent cells without the ability of proliferation provide the foundation for aging (Yuan et al., 2020b). Since proliferation is tightly controlled by both oncogenic and tumor-suppressing signaling networks, cancer and aging are intrinsically connected. It has been established that the incidences of most cancers rise sharply with age, yet the causal relationship still remains elusive. Aging and cancer share various hallmarks including genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, and cellular senescence (Hanahan and Weinberg, 2011; Kong et al., 2022; López-Otín et al., 2013).

Cellular senescence is a stable cell-cycle arrest program in response to various stresses including DNA/tissue damage or tumorigenesis-associated stresses, which is thought to be irreversible in terminating the further expansion of malignant cells. However, it has been reported that senescence-evoked intrinsic reprogramming promotes cancer cell stemness after the escape from a chemotherapy-induced senescent cell-cycle arrest (Duan et al., 2015; Milanovic et al., 2018). Cellular senescence usually exhibits heterogeneous phenotype associated with multiple biomarkers, including elevated expression of SA-β-Gal, p16, PAI-1, senescence-associated heterochromatic foci (SAHFs), DNA-SCARS and senescence-associated secretion phenotype (SASP). Senescence typically depends on the p53 and RB pathways and is associated with other effector programs including telomere shortening, persistent DDR, oncogene signaling, autophagy, SASP, and epigenetic gene regulation (López-Otín et al., 2013).

Oncogene-induced senescence (OIS)

Oncogenes, such as activated Ras, drive cell proliferation and survival while they can induce senescence. Senescent cells were found in the early stages of tumorigenesis, including prostate tumors, colon adenomas, astrocytoma, and neurofibromas with mutations in *RAS* or *BRAF* (Sieben et al., 2018). Ectopic expression of activated *RAS* or *BRAF* induces senescence in establishing a durable cell-cycle arrest (i.e., OIS) *in vitro* (Sieben et al., 2018). In the $Kras^{G12V}$-driven lung adenoma mice, which develop multiple lung adenomas (premalignant tumors) and lung adenocarcinomas (malignant tumors), there are abundant senescent cells as evidenced by readily detectable biomarkers for senescence, including p16 and SA-β-gal, in premalignant lung adenomas rather than malignant adenocarcinomas (Sieben et al., 2018). In a skin carcinoma mouse model, DMBA and TPA specifically induce *Hras* mutations and promote skin papilloma progression, concomitant with the accumulation of senescent cells via the p38-PRAK-p53 axis (Sun et al., 2007). DMBA/TPA-treated PRAK deficient mice exhibit shortened lifespan, accelerated skin carcinoma development with much reduced senescent cells (Sun et al., 2007). In the *LSL-*$Kras^{G12D}$ mice model, *Gata4* knockdown inhibits *Wnt7b* transcription and induces senescence in tumors (Gao et al., 2019). Knock-out of autophagy gene *Atg7* leads to the increase of mitochondria and ROS, thus promoting cell senescence, whereas the recovery of autophagy can inhibit cell senescence (White et al., 2021). Knockout of the autophagy-promoting gene, *Atg7* or *Atg5*, leads to accumulation of dysfunctional mitochondria, resulting in genomic instability, cellular senescence and blockage of $Kras^{G12D}$-driven lung tumor development (White et al., 2021). Together, these results strongly support the notion that OIS is a cellular defense program to block oncogene-driven tumorigenesis and that cancer development relies on overcoming or bypassing cellular senescence (Figure 9).

DNA Damage-induced senescence (DIS)

Genome integrity is constantly threatened by a variety of genotoxic insults including radical oxygen and nitrogen species and other endogenous and exogenous harmful reagents such as radiation and numerous chemicals (Di Micco et al., 2021). The erroneous repair can result in mutations potentially leading to altered gene functions, which in turn promotes cancer development, while severe or persistent DNA damage can induce cellular senescence (DIS) and apoptosis, eventually resulting in tissue degeneration and aging. Deficiency in NER is associated with an increased risk of various cancers (Di Micco et al., 2021). The XPF-ERCC1 dimer is required not only for NER but also for repairing cytotoxic interstrand crosslinks (ICLs) that can give rise to double-strand breaks (DSBs). XPF-ERCC1-deficient mice exhibit dramatic progeroid symptoms including aging-like skin, liver, bone marrow abnormalities and decrease of lifespan (Di Micco et al., 2021). These studies demonstrate that dysfunctional DNA damage repair is vital in both cancer development and the aging process.

p53 at the center stage of cancer and aging

TP53 is a vital tumor suppressor gene in regulating the development of cancer and aging. *TP53* mutations are found in more than 50% of human tumors and cancers (Petitjean et al., 2007). The incidence of lymphoma in p53 deficient mice was more than 70% at 6 months of age (Papazoglu and Mills, 2007). In *KRAS* mutated human pancreatic cancer and colon

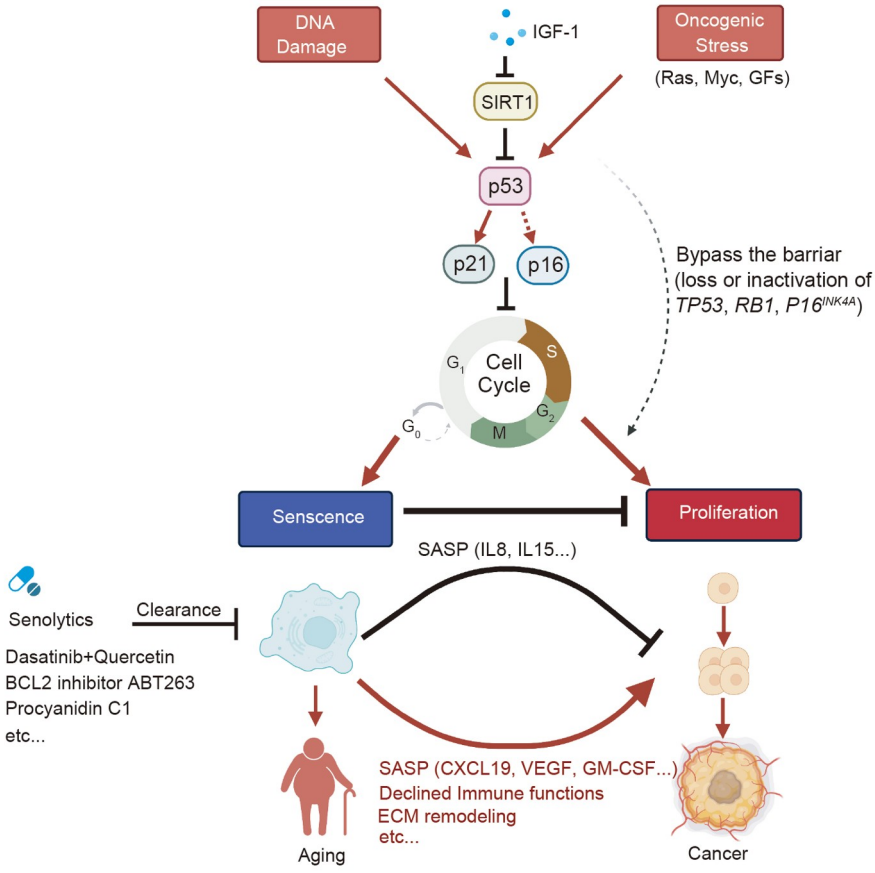

Figure 9 p53-Mediated senescence is at the center stage of aging and cancer. Stress-mediated p53 activation leads to cell cycle arrest, and consequently cellular senescence, resulting in blockage of cell proliferation. Cellular senescence has both pro-proliferation and anti-proliferation functions by affecting various aging/cancer-associated processes through regulation of cell cycle, immune system, inflammation and ECM remodeling. Senolytics-mediated selective elimination of senescent cells impacts both aging and cancer development.

cancer, the *TP53* mutation rate is 64% and 59%, respectively (https://www.cbioportal.org/), indicating that loss of *TP53* function together with activation of *RAS* proto-oncogene is essential in driving cancer development. Abundant evidence indicates that p53 is a key regulator in cellular senescence and aging. Abnormal expression of transcriptionally active p53 in mice leads to premature aging with nearly 30% shorter lifespan than wild-type mice (Tyner et al., 2002). While IGF-1 is an important growth factor promoting tumorigenesis, the defective IGF-1 signaling leads to extended lifespan in *C. elegans*, *Drosophila*, and mice (Papazoglu and Mills, 2007). It has been shown that IGF-1 induces cellular senescence via inhibition of SIRT1 and consequently p53 activation (Tran et al., 2014), suggesting that the aging role of IGF-1 is dependent on the SIRT1-p53 pathway. p53 not only antagonizes oncogenic transformation but also orchestrates non-cell-autonomous responses to DNA damage by mediating clearance of damaged cells through the innate immune system, which illustrates how the cellular senescence program can act together with the innate immune system to limit tumor growth. Importantly, p53 can modulate the SASP of senescent cells and subsequent impacts on immune cells. The SASP of p53-activated hepatic stellate senescent cells includes IL-6, ICAM-1 and IFNγ, which skew macrophage polarization toward a tumor-inhibiting M1-state, while p53-suppressed cells show elevated secretion of IL-3, IL-4 and IL-5, which stimulate polarization of macrophages into a tumor-promoting M2-state (Lujambio et al., 2013).

The role of SASP in tumor progression

Cellular senescence can lead to a continuous increase in the secretion of various inflammatory cytokines called SASP (Figure 9), and the continuous expression of these factors can drive the chronic inflammatory response, namely inflammatory senescence (Fane and Weeraratna, 2020). In a recent study of serum immune factors in a cohort of nearly 1,000 people, the inflammatory clock of aging (iAge) is shown to be associated with longevity in centenarians (Sayed et al., 2021). The main contributors to iAge include CXCL9, VEGF, EOTAXIN, MIP-1α, LEPTIN, IL-1β, IL-5, IL-6, IFN-α, IL-4, TRAIL, IFN-γ, CXCL1, IL-2, TGF-α, PAI-1

and LIF (Sayed et al., 2021). CXCL9 plays an important role in the development of colon cancer, lung cancer and prostate cancer (PCa) (Tokunaga et al., 2018). CXCL9 can promote the growth of PCa by inhibiting T cell secretion of IL-6 and TGF-β (Tokunaga et al., 2018). VEGF is also an important SASP, which plays an important role in promoting tumor cell proliferation, migration and tumor growth-related angiogenesis (Fane and Weeraratna, 2020).

It has been reported that the activation of SASP is directly linked to immune-mediated clearance of senescent cells. Endometrial stromal cells (ESCs) enter senescence during decidualization in an IL-8-dependent manner, while IL-15 activates uterine natural killer (uNK) cells to selectively clear senescent decidual cells through granule exocytosis (Fane and Weeraratna, 2020). Notably, in mouse models of PDAC, therapy-induced senescence triggers SASP-dependent vascular remodeling, which in turn facilitates chemotherapy uptake and efficacy and promotes T cell infiltration into PDAC lesions (Fane and Weeraratna, 2020), suggesting that senescence not only can stop the cell cycle to block proliferation but can also create susceptibilities to otherwise ineffective chemo- and immunotherapies through SASP-dependent effects on the tumor vasculature and immune system.

Role of ECM (extracellular matrix) in senescence and tumor progression

Expression of ECM changes vastly with age. The increase of age-related SASP and other aging matrix components leads to the remodeling of ECM and the increase of hardness in the microenvironment matrix (Fane and Weeraratna, 2020). The degree of malignancy of lung cancer is positively correlated with the increase of age, ECM strength, crosslinking and collagen density. In contrast, ECM remodeling in breast tissue can play both carcinogenic and anticancer roles (Bissell and Hines, 2011). ECM remodeling-mediated physical changes in the skin are the most visible signs of aging, which has been reported to facilitate melanoma metastasis. HAPLN1, a hyaluronic and proteoglycan link protein secreted by young dermal fibroblasts, is lost in aged fibroblasts. Age-related loss of HAPLN1 promotes melanoma cell motility and inhibits the migration of T cells (Kaur et al., 2019). Furthermore, treatment of recombinant HAPLN1 to aged fibroblast ECMs can reduce endothelial permeability via modulation of VE-cadherin junctions and limited visceral metastases (Fane and Weeraratna, 2020).

The effects of the immune system on aging and tumor progression

Immune senescence promotes tumorigenesis. Immune functions decline with the increase of age, manifested as thymus atrophy, decreased primary T cells and dysfunction of memory T cells, leading to the decline of effector immune cell subsets and overall immune function (Fane and Weeraratna, 2020). Analysis of the immune systems of the aging mice showed that expression of programmed cell death 1 ligand 1 (PDL1) and aging-related β-galactosidase is increased in immune cells in the dermis (Fane and Weeraratna, 2020). The incidence of *Hras*-induced skin cutaneous squamous cell carcinoma (SCC) is much higher in older mice (18–22 months of age) than in younger mice (2–4 months of age) (Fane and Weeraratna, 2020).

Perspectives on aging intervention strategies in tumor therapy

Overcoming cellular senescence is pivotal for the development of malignant tumors. Thus, re-senescence of tumor cells has been postulated as a feasible therapeutic strategy (pro-senescence therapy). Pro-senescence therapy (including telomerase inhibitors, CDK inhibitors, and the p53 targeting drug, Nutlin3a or APR-246) is designed to restore cellular senescence to tumor cells that have overcome OIS. For instance, treatment with CDK4 inhibitor PD0332991 leads to a significant increase in the SA-β-gal activity and γ-H2AX expression and inhibition of tumor growth in $Kras^{G12V}$-driven lung cancer mouse model (Puyol et al., 2010). JNK specific inhibitor SP600125 promotes premature aging of cancer cells by specifically activating Bcl-2-ROS-DDR signaling (Lee et al., 2010). However, many treatments for DNA toxicity lead to cellular senescence of normal somatic cells, which is called therapy-induced senescence (TIS) (Calcinotto and Alimonti, 2017). SASP produced by TIS may further lead to tumor proliferation and migration as well as blood vessel formation (Ferrara et al., 2003; Siersbæk et al., 2020). Recent studies have shown that targeted elimination of senescent fibroblasts in the p16-3MR mouse model can reduce short-term and long-term side effects related to chemotherapy cytotoxicity and suppress cancer recurrence and metastasis (Demaria et al., 2017). Therefore, effective removal of senescent cells may avoid the toxic and side effects of pro-senescence therapy.

Senolytics, such as D (Dasatinib)+Q (Quercetin) or ABT-263 (a selective BCL2 inhibitor), can specifically eliminate senescent cells by targeting survival pathways, including the Bcl-2/Bcl-XL, p53/P21, PI3K/AKT (Kirkland and Tchkonia, 2020). Thus, senolytics is postulated as a putative strategy for cancer therapy (Kirkland and Tchkonia, 2020). In one scenario, senescent hepatic stellate cells (HSCs) promote the growth of liver cancer cells through senescence-related secretion. The tumor-bearing mice treated with senolytics, D+Q or ABT-263, exhibit reduced aging HSCs resulting in inhibition of liver cancer progression (Kirkland and Tchkonia, 2020). However, in xenograft studies conducted with HCC cells inoculated in athymic nude mice, D+Q appears to have acute pro-tumorigenic effects (Kovacovicova et al., 2018). Serine protease inhibitor Kazal type I (SPINK1), a

SASP factor produced in human stromal cells after genotoxic treatment, promotes cancer cell aggressiveness. Targeting SPINK1 with a specific antibody significantly promotes tumor responses to mitoxantrone (Chen et al., 2018a). A recent study reported that procyanidin C1 (PCC1), a polyphenolic component of grape seed extract, inhibits SASP and selectively kills senescent cells via ROS accumulation in the cytoplasm (Xu et al., 2021). PCC1 combined with chemotherapeutic agents can eliminate senescent cells, promote cancer cell apoptosis, reduce tumor size, and ultimately improve the overall therapeutic effects (Xu et al., 2021).

Summary

It is now increasingly clear that cellular senescence has multiple and complex functions that are fundamentally important in cancer development and in the aging process. On the one hand, cellular senescence can function as a barrier to block oncogene-induced proliferation. On the other hand, cellular senescence can have tumor-promoting or tumor-inhibiting functions through SASP, inflammation and immune system. Hence, rather than clearance of senescent cells, identifying and targeting the pro-cancer SASP could be a more effective strategy for cancer therapy.

The features of organ aging

Aging is associated with alterations in tissue homeostasis and a decline in tissue functions. Common and different alterations in morphology, functions, molecular markers and drivers have been discovered in specific types of cells, tissues and organs. In this chapter, we present the general and specific characteristics of aged tissue/organs in multiple systems through the body and explore their etiology and interventions, in pursuit of potential therapeutic targets for aging-related diseases.

Vascular aging

A blood vessel is composed of tunica intima, tunica media, and tunica adventitia. The intima is a barrier between blood and vessel, including endothelial cells (ECs) and base membrane. The media hosts smooth muscle cells (SMCs), and adventitia is consist of connective tissue where fibroblast cells reside (Tian and Li, 2014) (Figure 10). The vascular system provides oxygen and other nutrients including active substances to all body cells and takes away carbon dioxide and wastes in tissues, suggesting vessels are essential for maintaining homeostasis *in vivo* (Schaum et al., 2020). Because vascular aging has been known as an independent risk factor for multiple diseases, theoretically, braking vascular aging process has become a reasonable tool to achieve the goal of reducing age-related diseases and successful healthy aging. Here we review the general characteristics of vascular aging and explore its etiology and interventions.

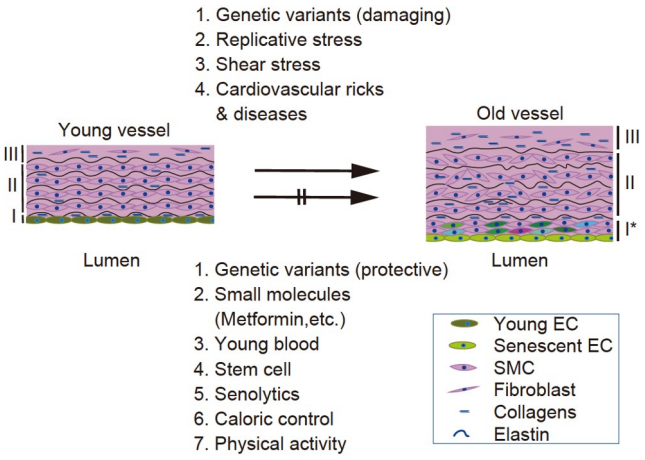

Figure 10 Vascular Aging and Intervention. The left side presents a young blood vessel with three layers, including I for tunica intima, II for tunica media, and III for tunica adventitia, respectively. The right side is an aged vessel with thickened intima (I*).

Characteristics of vascular aging

Vascular aging presents a set of changes in morphology, functions, and molecular markers as discussed below.

Morphologically, an aged vessel exhibits a few of changes that distinguish it from the young vessel (Bonithon-Kopp et al., 1996; Jani and Rajkumar, 2006). For example, it has an enlarged vascular lumen, diffused and thickened intima layer that consists of transformed cells (Figure 10, I*), and increased collagen deposition (Nakashima et al., 2002). In addition, the elastin loses curliness and becomes fractured. The arrangement of SMCs becomes disordered (Figure 10). These together are the jigsaw puzzle pieces for the morphological alterations of the aged vessels (López-Otín et al., 2013).

Functionally, vascular stiffness is increased, the systolic blood pressure is elevated, and vascular responses to stimuli is changed differentially with increased ages (Reece and Hulse, 2013). For example, it is more sensitive to vasoconstrictors, such as angiotensin II and serotonin (Vanhoutte, 1988), while becomes desensitized to dilators, such as acetylcholine (Vanhoutte, 1988). Furthermore, the ability for angiogenesis is reduced in the aged tissues when exposed to the hypoxia condition (Rivard et al., 1999). Functional vascular aging represents a net effect of vascular cell senescence and the renewal of those senescent cells.

Cellular senescence contributes directly to vascular aging. During vascular aging, telomere attrition and the increased expressions of other senescent markers, such as p53, $p21^{WAF1/Cip1}$ (CDKN1A), $p16^{INK4A}$ (CDKN2A), ROS and the genes related to SASP are observed (Katsuumi et al., 2018). Additionally, an array of genes related to vascular function

are also changed in their expressions, presenting as functional markers to measure vascular aging. For instance, the expression of endothelial nitric oxide synthase (eNOS) is decreased in aged vessel and becomes one of the commonly used markers in the field (Tian and Li, 2014).

Etiology of vascular aging

The causes of vascular aging have been linked to various factors. For instance, genetic background, replicative stress, shear stress of blood stream, cardiovascular risk factors and diseases are all shown to promote vascular aging.

One of the well-known theories to explain aging process is "programmed aging" (Trubitsyn, 2020). If the theory is correct, there should be a set of genes that control the aging process chronologically. Although the theory faces various challenges, the direct evidence that genetic components contribute to aging is from human progeria syndrome caused by genetic mutations. The sufferers exhibit massive age-related vascular disorders (Singh et al., 2019), such as atherosclerosis, suggestive of an inter-tissue coordination during aging processes. With cutting-edge technologies, such as genome-wide association and deep-sequencing, a dozen of genes are found to be associated or functionally contribute to tissue or organismal aging including longevity, a model for successful healthy aging, and vascular aging, including sirtuin 1 (*SIRT1*), forkhead box O1A (*FOXO1A*), forkhead box O3A (*FOXO3A*), adrenoceptor beta 2 (*ADRB2*) and apolipoprotein E (*APOE*). These genes regulate both vascular functions and aging (Castellano et al., 2003; Kane and Sinclair, 2018; Li et al., 2009; Yan et al., 2019b).

It has been reported that normal human diploid fibroblasts reduce replicative potential and have limited passages *in vitro* (Hayflick, 1965). Shortened telomeres and loss of proliferative potential are the nature of replicative senescence (Collins and Brunk, 1976). Similar to fibroblasts, vascular ECs and SMCs also present declined replicative potential during subculture (Minamino and Komuro, 2007). The normal ECs may be quiescent *in vivo*. However, ECs, when exposed to stresses, can be activated and become proliferative to replace the impaired or dysfunctional ECs (McDonald et al., 2018; Schwartz and Benditt, 1977). Notably, studies have shown that the replication rate of aortic endothelium decreases from a maximum of 13% at birth to 0.1%–0.3% at 5–6 months of age in the normal rats (Schwartz and Benditt, 1977), which illustrates the decreased replication rate of ECs and may explain the increased susceptibility to atherosclerotic diseases with aging. These data suggest that the replicative stress is an important cause of vascular aging.

Facing the strong shear stress of blood flow, ECs especially at branches become exhausted in proliferative capacity as these cells need to proliferate to maintain vascular integrity and functions. The shear stress promotes endothelial cell senescence (Tian and Li, 2014). Furthermore, shear stress affects the production of NO and secretion of prostacyclin in vessel, exacerbating vascular function and aging (Baeyens, 2018).

Cardiovascular risk factors and diseases, such as hypertension, hyperglycemia, and hyperlipidemia, promote endothelial dysfunction, increased vascular stiffness and susceptibility to atherosclerosis (McCarthy et al., 2019). Additionally, smoking and higher levels of alcohol consumption are associated with increased risk of cardiovascular disease by increasing ROS production and reducing NO activity (Vanhoutte et al., 2017). Notably, long-term exposure to negative emotions contributes to vascular aging and age-related vascular diseases as well (Balog, 2018). These risk factors of cardiovascular diseases accelerate vascular aging significantly, although they can be avoided and treated.

Intervention of vascular aging

Vascular aging may be a common target to alleviate the age-related vascular diseases and extend healthspan. Promisingly, recent studies have found some avenues to slowdown vascular aging (Figure 10). First, a number of small molecules, such as nicotinamide mononucleotide (NMN), NR, metformin, resveratrol, spermidine, and so on, are shown to be effective in prevention of age-related vascular diseases (Das et al., 2018; El Messaoudi et al., 2011; LaRocca et al., 2013; Michiels et al., 2016). Second, studies have shown that transferrable factors in young blood reverse degenerated phenotypes associated with ages. Third, senolytics and stem cell therapy are found to delay vascular aging and postpone age-related vascular diseases (de Magalhães et al., 2017). Finally, physical activity and caloric control are shown practical to brake vascular aging in animal models and human populations (De Miguel et al., 2021; Rossman et al., 2017). However, the long-term effect and safety, particularly in human, need more careful investigations.

Summary

Vascular aging is an important risk factor to organismal aging and aging-related diseases. An aged vessel presents unique changes morphologically and functionally. Vascular aging is intervenable in practice, thus, delay of vascular aging has significant clinical implications.

Cell-type specific brain aging

As with other organs, the functional capabilities of brain decline progressively during aging, which particularly manifests as decrements in learning and memory (Mattson and Arumugam, 2018). Learning and memory function relies on modifications of synaptic transmission, also known as synaptic plasticity, which alters dynamically in response to

environmental stimulation. Both hippocampus and cortex are brain regions critical for learning and memory (Preston and Eichenbaum, 2013). Recent studies also reveal that cerebellum has motor-independent learning and memory activities (Bellebaum and Daum, 2007; Likova et al., 2021).

Brain cells, including neuronal cells and non-neuronal cells such as astrocytes, undergo cellular senescence during aging. Brain cells possess some common cellular senescent features, and each individual cell type also possesses unique senescent features (Mattson and Magnus, 2006; Salas et al., 2020).

Neuronal aging

Neurons in aged mice possess common cellular senescent features, including elevated expression of phosphorylated p38/MAPK (p-p38), γH2AX, and increased SA-β-Gal activity, and accumulation of lipofuscin (Jurk et al., 2012). Neurons are post-mitotic cells (permanently in the G0 phase of the cell cycle), and neuronal senescence must rely on other foundations other than proliferation arrest. Some studies have reported minimal to no loss of neurons in both the neocortex and the hippocampus of the brain during normal aging, whereas other studies have observed some neuronal loss in the cerebellum and substantia nigra with age (Morrison and Baxter, 2012; Wang et al., 2021c; Woodruff-Pak et al., 2010; Zhang et al., 2021c). Although cerebellum has been shown also having learning and memory functions, hippocampus and cortex are major brain regions responsible for memory formation, therefore, the memory decline observed in aged animals is unlikely due to neuronal death, and more likely due to the breakdown of neuronal circuits and disruption of brain connectivity.

Pyramid neurons in the neocortex and hippocampus possess extensive apical and basilar dendritic trees, which integrate information from both excitatory and inhibitory synaptic inputs. Neurons in layer V of the prefrontal cortex exhibit progressive regression in dendritic arbors during aging. In addition, these neurons also show decrease in dendritic length and complexity with age (de Brabander et al., 1998; Shimada et al., 2014). Similarly, hippocampal CA1 neurons also show age-related regression of apical dendrites and decreased dendritic complexity in aged animals (Markham et al., 2005).

Spines are the primary sites of excitatory and inhibitory synapses and of forms of synaptic transmission and synaptic plasticity. Therefore, changes in spine size, shape, density or its distribution along the dendritic shafts may affect synaptic events critical to learning and memory function (Morrison and Baxter, 2012). In comparison to changes in neuronal structure, changes in spine density and morphology are more drastic. Decrease in spine densities are reported in almost all levels of dendrites, with decrease in both the spine densities and the number of total spines in the basal and apical dendrites during aging (Kabaso et al., 2009; Page, 2002). Similarly, a reduction in the number of perforated synapses in hippocampal dentate gyrus (DG) area is reported (Hara et al., 2012). In addition to DG, hippocampal CA3, a brain region believed to be particularly sensitive and vulnerable to aging, also shows decreased synapses (Adams et al., 2010; Zhang et al., 2021c). These findings suggest that hippocampal and cortical connectivity is more susceptible to aging, therefore these regions are prone to the age-induced damages. Synaptic proteins have been implicated in mechanisms of synapse plasticity, and learning and memory. Aged animals exhibit reduced synaptophysin in the hippocampus (Smith et al., 2000). Decrease in synaptic protein SNAP25 is associated with faster cognitive decline (Bereczki et al., 2016). Proteomic studies have comprehensively examined the levels of synaptic proteins, and found that cognitive impairment is associated with reductions in multiple synaptic proteins (Bereczki et al., 2018; Wingo et al., 2019). Moreover, increase in synaptic proteins in the hippocampus can rescue memory deficits in aged mice (Bustos et al., 2017).

Pre- and postsynaptic firing of action potentials either strengthen or weaken signal transmission, leading to long-term potentiation (LTP) or long-term depression (LTD) (Glasgow et al., 2019). Hippocampal Schaffer collaterals CA1 synapses show reduced amplitude of excitatory postsynaptic potential (EPSP), suggesting a loss of functional synapses in the CA1. Similarly, decreased EPSP amplitude is also found in DG region of aged hippocampus, in addition, aged DG also exhibits lower amplitude of fiber potential at the perforant path (DG granule cell synapse). Taken together, these changes in electrophysiological properties confirm the anatomical changes in structural plasticity.

Astrocytic aging

Astrocytes represent the largest population of glial cells in the brain and constantly provide support for surrounding neurons to maintain a healthy environment (Allen and Barres, 2009). The expression of glial fibrillary acidic protein (GFAP), the main constituent of astrocyte intermediate filament and also a hallmark of reactive astrocytes, is increased with age, suggesting an occurrence of astrogliosis, which is a response of activated astrocytes (Liddelow et al., 2017; Sofroniew, 2014). In addition to *Gfap*, a set of upregulated genes in aged astrocytes is tightly associated with reactive state of astrocytes (Boisvert et al., 2018; Clarke et al., 2018). In support of this, astrocytes phenotypically change their morphology from long and slender processes to short and stubby processes during aging (Jyothi et al., 2015; Robillard et al., 2016), also supporting a transition from resting state to active state during aging. The activation of astrocytes in the hippocampus leads to impaired memory functions in rats (Li et al., 2020c).

In adult brain, neurons rely heavily on astrocytes for

supply of metabolic substances, such as cholesterol. Metabolic changes induced by age in astrocytes subsequently lead to metabolic disturbance in neurons (Cotto et al., 2019), and may eventually affect neuronal functions. For example, the biosynthesis capacity is reduced in astrocytes during aging (Boisvert et al., 2018). Moreover, APOE, the predominant carrier for cholesterol transport from astrocytes to neurons, also shows a region-specific change during brain aging (Martin et al., 2010). These findings indicate that both cholesterol biosynthesis and transport are impaired in aged brain. Functionally, aged *ApoE* knockout mice, but not young knockout mice, show learning and memory deficits (Fuentes et al., 2018), suggesting that aging is an essential confounder for *ApoE*-mediated memory function. Consistently, *ApoE* knockdown in astrocytes of adult mice also leads to defective memory through miRNA-mediated transcriptional suppression of genes related to memory formation (Li et al., 2021e). RNA-binding proteins (RBPs) are key regulators in modulating gene expression by controlling mRNA turnover and translation (Glisovic et al., 2008; Pullmann et al., 2007). A finding from our group demonstrates that RNA binding protein SFRS11 shows substantially decreased abundance in aging astrocytes and loss of SFRS11 in astrocytes leads to not only memory impairment, but also transition of astrocytes to active state (Raihan et al., 2019), suggesting a role of RBPs in aging-induced activation of astrocytes.

Microglial aging

Microglia are the resident immune cells in the brain and constitute around 10% of brain cells (Salas et al., 2020). Microglia in aged brains show increased inflammatory profile, including proinflammatory cytokines such as TNFα, IL-1β and IL-6 (Sierra et al., 2007). In addition, microglia in aged brains tend to be activated, as evident by de-ramified morphology (Hwang et al., 2008). In supporting of this, a striking buildup of lipid droplets in microglia with aging in mouse and human brains is observed. These lipid droplet-accumulating microglia show defective phagocytosis, produce high levels of reactive oxygen species and secrete more proinflammatory cytokines (Marschallinger et al., 2020). Disruption in the balance between proinflammatory and anti-inflammatory molecules released by microglia is tightly associated with synaptic plasticity (Golia et al., 2019). Inhibition of proinflammatory molecule release in the hippocampus prevents microglia from activation, enhances synaptic plasticity and cognitive function (Feng et al., 2019). Transcriptome analysis reveals that microglia show both age-dependent and region-dependent changes in aged brains (Grabert et al., 2016), suggesting that microglia are differentially sensitive to aging. In brief, with age, microglia change their morphology into dystrophic, de-ramified and spheroid shape, which is also a factor in accelerating brain aging progression.

Summary

Taken together, the mammalian brain is a complex organ, with multiple cell types performing a variety of diverse functions. Aging not only induces common changes in molecular features, but also drives distinct changes in each cell population (Figure 11). More research is needed to understand and modify brain aging process.

Molecular mechanism of pulmonary aging

Human lung maturation and function peak between the age of 19–25, and remain steady up to the age of 35. However, the pulmonary function declines thereafter gradually, and advanced aging underlies progressive deterioration of the lung function in otherwise healthy individuals. Aging-related pulmonary dysfunction includes impaired gas exchange, reduced mucociliary clearance, and immune defense disorders that predispose to infections (Sharma and Goodwin, 2006). With a large and growing aging population, understanding the aging process in the lung is necessary to provide optimal care to our aging population.

Structural and functional changes of aging lung

The structural basis of pulmonary aging and related functional decline involve decreased cellularity and increased ECM in a disordered manner, causing compromised tissue elasticity and reduced gas transfer. The disordered network of collagen fibers coil around the alveolar ducts and adjacent alveoli, with decreased elastic fibers and increased collagen content, resulting in alveolar duct dilation and homogeneous enlargement of alveolar air spaces (Godin et al., 2016; Hsia et al., 2010; Skloot, 2017). Gradual aging-associated loss of pulmonary elastic recoil pressure impairs expiratory flows and volumes, with compromises of forced expiratory volume in one second (FEV1), forced vital capacity (FVC), and FEV-1/FVC ratio (Sharma and Goodwin, 2006). It was reported that FEV1 loses approximately 30 mL every year beginning after 30 years of age (Lowery et al., 2013). FVC and increased functional residual capacity (resting lung volume) also decline gradually with age (Janssens et al., 1999). As the most important function of the lung, gas exchange across the alveolar capillary membrane, measured by diffusing capacity of carbon monoxide (DLCO) for lung volume and alveolar ventilation, becomes weakened with age (Sharma and Goodwin, 2006). Furthermore, in the characters of respiratory pattern in the elderly, the lower tidal volumes and higher respiratory rates (Janssens, 2005) in association with narrowing peripheral airway calibers (Brusasco et al., 2015) appear to be mediated by decreased tensile strength of inspiratory/expiratory respiratory muscles and thus increased stiffness of parenchymal and vessel compartments (Lalley,

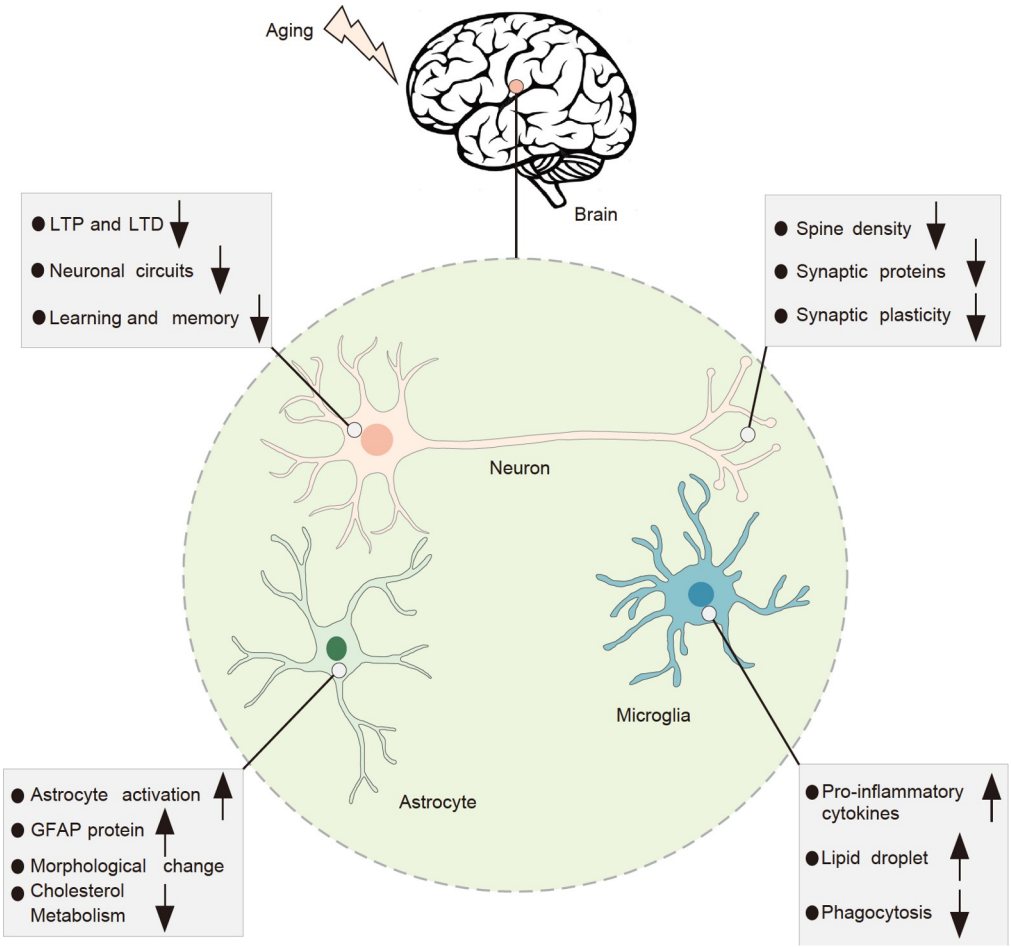

Figure 11 Cell-type specific aging in the brain. The cell-type specific hallmarks in neurons, astrocytes and microglia during aging. During aging, neurons show decreased spine density and synaptic proteins, resulting in declined synaptic plasticity and impaired learning and memory. Astrocytes show increased GFAP expression and astrocytic activation with age, which is accompanied by dysregulated cholesterol metabolism and morphological changes. With age, microglia exhibit accumulated lipid droplets and increased proinflammatory cytokines, leading to decreased phagocytosis capacity. LTP, Long-term potentiation; LTD, Long-term depression; GFAP, glial fibrillary acidic protein.

2013; Sicard et al., 2018). While none of these changes in pulmonary structure and function with age is sufficient to cause symptoms, the aged lung appears more susceptible to damage incurred by environmental stresses, such as viral infection and cigarette smoking.

Cellular mechanisms of pulmonary aging

Human lungs are composed of a unique set of multiple cell types in the epithelia covering respiratory airways and pulmonary alveoli, which face ongoing chemical, mechanical, biological, immunological and xenobiotic stress over a lifetime (Schneider et al., 2021). In the respiratory tract, numerous structural and functional changes occur with pulmonary aging (Cho and Stout-Delgado, 2020). As a vital barrier between the environment and internal tissues in the airways, the respiratory epithelium is the first line of defense against inhaled foreign materials (Knight and Holgate, 2003). Lining the trachea, bronchi, and bronchioles by a ciliated pseudostratified epithelium (Ball et al., 2022), the ciliated cell population increases with age, leading to an altered ratio of club-to-ciliated cells in the aged mouse airways, whereas the basal and club cells decrease in number with age, potentially underlying decreased ciliary beat frequency, decreased mucociliary clearance, and increased predisposition of the elderly to pneumonia (Angelidis et al., 2019; Bailey et al., 2014). By long-term EdU incorporation analysis and immunohistochemistry, it has been shown that bronchiolar cell density remains stable with age, but inferred rates of bronchiolar club progenitor cell self-renewal and differentiation are reduced significantly, suggesting an overall slowdown in cellular turnover (Figure 12) (Watson et al., 2020).

The alveolar epithelium, especially the type I alveolar epithelial cells (AEC1) and cuboidal AEC2 are vulnerable to aging-related sustainability with declined cellularity and increased interstitial deposition (Wu and Song, 2020). While AEC1 cells are terminally differentiated occupying over 85% of the alveolar epithelial cells, AEC2 represents the

local alveolar stem cell population serving as the predominant regenerative reserves for renewal and AEC1 damage repairs by proliferation and differentiation (Olajuyin et al., 2019; Wang et al., 2020b). In addition, age-related decreases in the ratio of proliferating versus apoptotic AECs have been exemplified (Walski et al., 2009), and the regenerative, self-renewal and differentiation capacities of AEC2 shown to be declined during aging (Ortega-Martínez et al., 2016; Schulte et al., 2019; Wang et al., 2020b; Wansleeben et al., 2014; Watson et al., 2020). Moreover, multiple lines of evidence indicate that AEC2 cell exhaustion mediates the development of pulmonary fibrogenesis (Wu et al., 2020a).

In conjunction with the airway- and alveolus-resident lung epithelial cell aging, alveolar macrophages (AMs) (Gomez Perdiguero et al., 2015) and peribronchial interstitial macrophages (IMs) (Gibbings et al., 2017), which are central to orchestrating the pulmonary immune response, have been demonstrated to behave abnormally with age. A decline in the levels of AMs occurs in the respiratory tract during aging (Wong et al., 2017). In addition, a number of functional deficits—including failures in phagocytosis and scavenging capacity, refractory activation to interferon (IFN) have been observed (Albright et al., 2016). Additional to instigating local immune cell disorders, inflammatory cell infiltration takes place in aging-related disorders, characteristic of cell senescence-association low-grade inflammation (SALI)—involving inflammatory cell infiltration and SASP (Alder et al., 2015; Chen et al., 2015b). Furthermore, pulmonary interstitial fibroblasts operate as an important cell population engaged in cellular remodeling, by mechanisms under the conditions of cellular replicative senescence and senescence-associated differentiation disorder (SADD) linking to the age-related pulmonary disease (Yanai et al., 2015).

Telomere dysfunction mediates pulmonary cellular senescence

Mechanistic investigations have shown that senescence-associated stem cell exhaustion is elicited by shortened telomeres, DDR, epigenetic alteration, oxidative stress response, and mitochondrial dysfunction (Hamsanathan et al., 2019; Hansel et al., 2020; Hernandez-Gonzalez et al., 2021; Liu et al., 2021b; Liu et al., 2019a; Wang et al., 2020b; Zhang et al., 2021d). Recent studies have further established that telomere dysfunction is causal to pulmonary cellular senescence (Naikawadi et al., 2020; Peters-Hall et al., 2020; Piñeiro-Hermida et al., 2020; Wang et al., 2020b; Zhang et al., 2021d).

Mice deficient of either *TERT* or the *telomerase RNA component* (*TERC*), lose telomerase function and thus telomere maintenance, entraining telomere shortening, AEC2 cell premature replicative senescence (Chen et al., 2015b; Wang et al., 2020b) and apoptosis in an TP53- and $p21^{CIP1/CDKN1A}$-dependent manner (Zhang et al., 2021d). In the animal models of telomerase deficiency, *TERT* knockout prompts pulmonary fibrosis after receiving low doses of bleomycin (Chen et al., 2015b; Povedano et al., 2015), or emphysema after chronic cigarette exposure (Alder et al., 2011). Correspondingly, mutations in *hTERT* and *hTERC* have been associated with telomere shortening and aging-related lung diseases, such as the mutations of V144M, R865C and R865H in *hTERC* (Armanios, 2012; Armanios et al., 2007; Fingerlin et al., 2013; Stanley et al., 2015; Tsakiri et al., 2007). Moreover, telomerase ectopic expression using AAV9 vectors illustrates a significant therapeutic effect on pulmonary fibrosis with improved lung function, lengthened telomeres, and increased proliferation of AEC2 cells (Povedano et al., 2018). In AEC2 cell-specific deletion of TRF2 and dysfunction of telomeres, cell senescence occurs and in turn causes an enhanced proinflammatory response with increased number of macrophages in the bronchoalveolar fluid (BALF), lung remodeling and pulmonary fibrosis (Alder et al., 2015; Povedano et al., 2015). Furthermore, a recent study showed that stress-induced catabolic degradation of TPP1 triggers reversible telomere uncapping and shortening, AEC2 stem cell senescence and pulmonary fibrosis (Wang et al., 2020b). Taken together, these investigations corroborate the fundamental importance of telomere maintenance in AEC2 stem cell renewal and proliferation, and telomere dyshomeostasis-induced AEC2 stem cell senescence in causing pulmonary premature aging and related diseases.

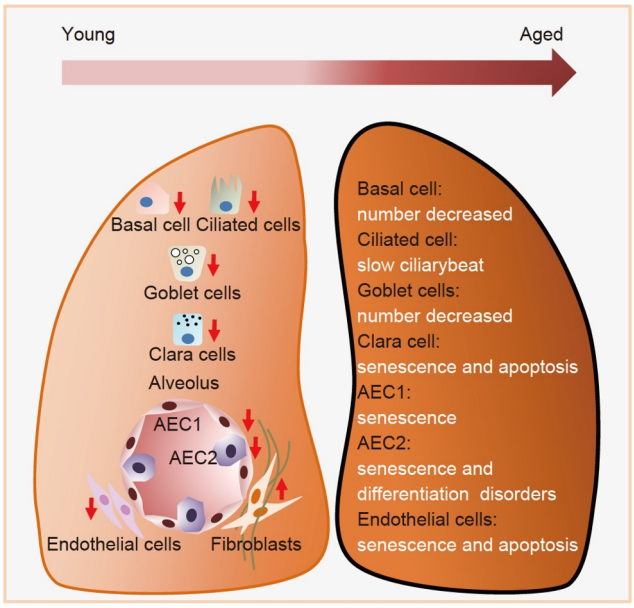

Figure 12 Cellular phenotype of pulmonary senescence. AEC1: type 1 alveolar epithelial cells. AEC2: type 2 alveolar epithelial cells—alveolar stem cells.

Summary

Pulmonary cellular senescence represents a significant risk factor of lung disease in older populations, through the mechanism of telomere dysfunction-mediated acceleration of AEC2 stem cell replicative senescence. However, the detailed mechanism of how age increases the risk of aging-related pulmonary diseases remains largely unclear. Additional research and advanced technology are needed to improve our understanding of the determinants of lung aging and the effects of aging on lung disease.

Cardiac aging

Cardiac aging is accompanied with a progressive decline in cardiac function and structure, due to the accumulation of cellular and tissue damage over time (Fontana, 2018). Here, we summarize the functional and structural features of cardiac aging, the recent reports on the molecular mechanisms of cardiac aging, and the potential interventions for cardiac aging-related diseases.

Characteristics of cardiac aging

The heart is composed of multiple types of cells such as cardiomyocytes, fibroblasts, endocardial cells, epicardial cells, pericytes, endothelial cells, smooth muscle cells, neurons and several types of immune cells (Litviňuková et al., 2020). Cardiomyocytes account for more than half of the weight of the heart and their main role is maintaining the circulation of cardiac systolic and diastolic functions. In aged heart, cardiomyocytes gradually decrease in number and increase in size (Triposkiadis et al., 2019). Moreover, cardiac aging is accompanied with the proliferation of cardiac fibroblasts and consequent accumulation of collagen deposition, often contributing the progression of cardiac fibrosis (Karamitsos et al., 2020). As fibrotic heart tissue is often stiffer and less compliant, this change subsequently leads to cardiac dysfunction and increased risk for heart diseases over time (Donekal et al., 2014). In otherwise healthy individuals, cardiac aging has often been associated with changes including left ventricular (LV) hypertrophy, LV wall thickening and decreased LV contractile function (Triposkiadis et al., 2019). In addition, cardiac aging is linked with decreased maximal heart rate, reduced maximal ejection fraction, and lower maximal cardiac output. These changes may lead to impaired cardiac reserve, leading to effort intolerance, frailty, and to some extent reduced quality of life in elder populations. Aging myocardium may also exhibit intrinsic electrophysiological changes that are regulated by the cardiac autonomic nervous system, thus increasing the risk of arrhythmias in the elderly (Chadda et al., 2018). Additionally, degenerative changes in heart valves are also observed with age as abnormalities such as severe aortic stenosis and mitral regurgitation are reported in approximately 10% of the population over the age of 75 (Rostagno, 2019).

As age goes on, degenerative changes at the cellular and tissue levels may further develop into various age-related heart diseases. Previous studies have reported that aging-related cardiomyopathy in 2-year-old mice is characterized by decreased LV function, increased fibrosis and cardiac hypertrophy (Yan et al., 2013b). During aging, the degeneration of cardiac structure and function also increases the susceptibility to heart failure. In addition, compared with younger subjects, the elderly population are more likely to develop myocardial infarction and suffers from subsequent cardiac dysfunction attributable to partial myocardial loss, replacement fibrosis, compensatory myocardial hypertrophy and maladaptive ventricular dilatation.

Mechanisms of cardiac aging

Many cellular and molecular alterations can contribute to adverse cardiac remodeling and dysfunction during cardiac aging, and the etiology of cardiac aging and related diseases involves multiple molecular mechanisms. Prevailing theories on aging place proteostasis imbalance as a central factor, manifested by the accumulation of misfolded proteins, decreased expression of chaperones, proteasomes, ribosomal and mitochondrial proteins, and upregulated proteins associated with oxidative stress (Kalfalah et al., 2015). In the heart, the loss of protein patency, due to genetically determined design flaws or environmental “wear and tear”, can overwhelm protein quality control (PQC), derailing the protein homeostasis. As such, the age-related progressive accumulation of misfolded or damaged proteins, and concomitant PQC failure can lead to proteotoxic stress, thus playing a vital role in various aging-related heart diseases (Vilchez et al., 2014).

Mitochondrial homeostasis is another essential factor for maintaining normal cardiac function, as the continuous contraction of the heart requires a large amount of ATP for energy supply. By comparison, mitochondrial dyshomeostasis leads to cardiac abnormalities as the highest mitochondrial mass in the cardiomyocytes among all the cell types rendering them susceptible to mitochondrial dysfunction and oxidative damage from ROS as byproducts of mitochondrial respiration (Zhang et al., 2022b). Mitochondrial homeostasis is tightly controlled by the dynamic coordination between mitophagy, mitochondrial biogenesis and mitochondrial dynamics (i.e., fission and fusion). The aging process is often accompanied by increased mitochondrial damage, which is mostly controlled through the selective scavenging of damaged organelles via mitophagy. Conversely, cardiac mitochondrial biogenesis is to some extent impaired in rodents and human with age. In particular, PGC1α, the master regulator of mitochondrial biogenesis, is expressed at lower levels in aged rodent and human myo-

cardium compared to their younger counterparts, whereas moderate overexpression of PGC-1α can inhibit the pathological remodeling of aged hearts and reduce apoptosis (Whitehead et al., 2018). In addition, cardiac aging disrupts the balance of mitochondrial fusion and fission in the heart, resulting in cardiomyocyte dysfunction (Youle and van der Bliek, 2012). Abnormally giant mitochondria have been observed in aging human myocardium, mainly due to more frequent mitochondrial fusion than fission.

Genetic damage that accumulates throughout life is also an important cause of cardiac aging. Telomere shortening is widely associated with cellular senescence and shortened telomeres have been discovered to impair cardiovascular function (Moslehi et al., 2012). *Terc*-knockout mice exhibit cardiac phenotypes including increased cardiomyocyte apoptosis and LV contractile dysfunction while overexpression of *Tert* improves ventricular function (Bär et al., 2014). In addition, telomere dysfunction also adversely affects cardiac mitochondrial function, further contributing to cardiac dysfunction. On the other hand, mice with genomic instability associated with defective nucleotide excision repair-deficient genes *Ercc1* and *Xpd* has been found to reproduce the features of aging in the aorta (Durik et al., 2012). Genomic instability in aging vascular SMCs has been shown to increase the expression of phosphodiesterase type 1 (PDE1), which subsequently impairs NO/cGMP signaling and induces endothelial dysfunction (Bautista Niño et al., 2015). Several studies have also revealed chromosomal lesions and mtDNA deletions in the PBMCs from the patients with coronary heart diseases, which are correlated with cardiac disease severity.

Growing evidence also suggests that epigenetic modifications can disrupt transcriptional programs related to oxidative stress, inflammation, angiogenesis, and cellular metabolism, thereby promoting maladaptive pathways and hallmarks of cardiac aging (Costantino et al., 2015). Monomethylation of histone H3 at lysine 4 (H3K4m) by the mammalian methyltransferase *Set7/9* regulates endothelial NF-κB signaling and is associated with persistent vascular inflammation (Paneni et al., 2013). In addition, histone deacetylation by *Sirt1* may also modulate age-related cardiovascular diseases as transgenic overexpression of *Sirt1* improves endothelial function in aged mice.

Interventions of cardiac aging

In-depth understanding of the pathogenesis of cardiac aging may immensely promote the development of targeted interventions for cardiac aging. As mitochondrial dysfunction and ROS are key factors in exacerbating cardiac aging and related disorders, they also make potentially attractive targets for intervening cardiac aging. In fact, the expression of mitochondrial-targeted antioxidant peptide has been shown to ameliorate hypertensive cardiomyopathy (Dai et al., 2011a). Additionally, in rodent models of cardiac aging, CR alleviates LV hypertrophy, attenuates cardiac apoptosis and fibrosis, and thus improves cardiac function (de Lucia et al., 2018). Besides numerous animal studies and preclinical investigations, multiple clinical studies based on the use of small-molecule compounds, targeted geroprotective drugs, different forms of exercise and so forth have also been completed or are being carried out for intervening cardiac aging and related diseases, some of which have shown the efficacy in improving the cardiac function of the elderly population (Table 1).

In recent years, cell-based therapies for aging-related cardiac diseases have also attracted great attention. Various types of cells have been shown to possess the potential for intervening cardiac aging, including pluripotent stem cells, MSCs, cardiac progenitor cells and PBMCs. In particular, stem cell-based therapies have been demonstrated to activate endogenous regenerative processes, including the recruitment of tissue-resident progenitor cells and the secretion of soluble factors (Rikhtegar et al., 2019). It has also been shown that the transplantation of cardiosphere-derived cells into aging rat hearts effectively enhance cardiac systolic and diastolic function (Grigorian-Shamagian et al., 2017). Another relatively new cell-based therapy employs antifibrotic CAR T cells by adoptive transfer or via *in vivo* reprogramming by modified mRNAs in T cell-targeted lipid nanoparticles to eliminate activated cardiac fibroblasts and reduce cardiac fibrosis, and thus restore the cardiac function after injury in mice, holding great promise as a therapeutic strategy to treat various aging-related cardiac diseases (Aghajanian et al., 2019; Rurik et al., 2022). Despite of some promising results from preliminary studies of various cell-based therapies, challenges remain such as low therapeutic efficacy, cell type selection, and potential safety concerns related to stem cell transplantation and tumorigenesis.

Summary

Overall, the underlying mechanisms of cardiac aging and related diseases are intricate and far from being fully understood. Still, continual elucidation of the potential molecular and cellular mechanistic insights of cardiac aging will facilitate the development of more therapeutic strategies to alleviate age-related heart dysfunction.

Bone function and aging-related diseases

Bone is a dynamic tissue that is continuously remodeled to preserve structural integrity of skeleton and protect visceral organs. Bone also provides homeostatic functions and serves as an important repository for 99% of total body calcium, whose inflow and outflow from bone is flat, with about five mmol turnover per day (Song, 2017). Derangements of calcium lead to hypercalcemia and hypocalcemia, which has

Table 1 Clinical trials for age-related heart diseases currently listed in https://clinicaltrials.gov/[a)]

Condition or Disease	Age	Intervention/ Treatment	Mechanisms and effects	Clinical Trail Identifier	References
Coronary artery disease	>60 years	Rapamycin	Inhibit mTOR activity; Suppress senescence-associated secretory phenotypes	NCT01649960	(Singh et al., 2016)
Coronary artery disease	Older (undisclosed specific range)	Ivabradine	Reduce heart rate	NCT02584439	(Fox et al., 2014)
Coronary artery disease	>75 years	Prasugrel Clopidogrel	Attenuate platelet inhibition	NCT01107912	(Erlinge et al., 2013)
Coronary artery disease	40–75 years	Ticagrelor	Fewer bleeding events; Reduce the rate of death from vascular causes, myocardial infarction, or stroke	NCT04999293	(Gimbel et al., 2020)
Coronary artery disease	40–75 years	Dietary Supplement: Aged Garlic Extract	Reduce IL-6, blood sugar levels and blood pressure; Inhibit coronary artery calcification progression; Reduce progression of atherosclerosis	NCT03860350	(Wlosinska et al., 2021)
Coronary artery disease	35–80 years	Dietary Supplement: Aged garlic extract and Coenzyme Q10	Inhibit the rate of progression of coronary calcification	NCT00860847	(Budoff et al., 2004)
Coronary artery disease	Older (undisclosed specific range)	Biological: blood sample withdrawn	Non-enzymatic post-translational modifications; Post-translational modifications derived products	NCT02857387	NA
Acute myocardial infarction	>50 years	Sildenafil	Increase left ventricular end-diastolic volume index	NCT01046838	(Andersen et al., 2013)
Acute myocardial infarction	70–82 years	OMega-3	Not reduce circulating prothrombotic microvesicles	NCT01841944	(Kalstad et al., 2021)
Atrial fibrillation	75–94 years	Warfarin Aspirin	Decrease the incidence of ischemic stroke	NCT01438580	(Wu et al., 2013)
Atrial fibrillation	>80 years	Du-176b	Prevent stroke or systemic embolism	NCT02801669	(Okumura et al., 2020)
Arrhythmia	>65 years	Dietary Supplement: DHA	Improve the heart rate variability	NCT00749307	NA
Heart failure	>60 years	Spironolactone	Reduce fibrosis, inflammation, thrombosis and congestion; Improve vascular function; Mediate cardiovascular protective effects; Slowing progression of heart failure	NCT02556450	(Pellicori et al., 2020)
Heart failure	>60 years	Spironolactone	Limit excessive extracellular matrix turnover; Block aldosterone receptors; Reduce the risk of both morbidity and death about heart failure	NCT00123955	(Daniel et al., 2009)
Heart failure	>60 years	ALT-711	Improve total arterial compliance in aged human with vascular stiffening	NCT00043836	(Zile et al., 2001)
Heart failure	>60 years	Exercise	Improve microvascular and/or skeletal muscle function; Improve peak and submaximal exercise capacity	NCT01113840	(Kitzman et al., 2013)
Chronic heart failure	>65 years	Aerobic endurance exercise training (ergometer)	Block ubiquitin-proteasome system activation; Improve left ventricular diastolic function	NCT00176319	(Gielen et al., 2012)
Chronic heart failure	60–70 years	Eccentric exercise	Increase REE and fat oxidation; Improve blood lipid profile	NCT01673958	(Paschalis et al., 2011)
Chronic heart failure	>65 years	Stretching and resistance training	Improve endothelial function	NCT00733161	(Ozasa et al., 2012)
HFpEF	>60 years	Behavioral: Exercise Dietary Supplement: Dietary Intervention	Increase peak V_{O2}	NCT00959660	(Singleton et al., 2022)
Normal aging heart	65–80 years	ALT-711	Improve aging-related LV stiffness moderately	NCT01014572	(Fujimoto et al., 2013)
Normal aging heart	60–75 years	Fenofibrate	Reverse the age-related decline in cardiac fat metabolism and mechanical function	NCT00627653	(Haldeman et al., 1999)
Normal aging heart	60–79 years	Ramipril	Inhibit angiotensin-converting enzyme; Reduce vascular stiffness; Ameliorate left ventricular function and energetics	NCT01504828	(Parikh et al., 2016)
Normal aging heart	70–87 years	Dietary Supplement: Selenium and ubiquinone (Q10) combined	Decrease oxidative stress and inflammation; Increase cardiac function; Reduce cardiovascular mortality	NCT01443780	(Alehagen et al., 2020)
Normal aging heart	50–79 years	Training	Rescue microvascular dysfunction	NCT02796976	(Streese et al., 2020)
Normal aging heart	>65 years	Exercise Tolerance	Improve cardiac function	NCT02779972	NA
Normal aging heart	>70 years	Aerobic interval training	Improve LV diastolic and systolic function	NCT00804518	(Molmen et al., 2012)

a) DHA, docosahexaenoic acid; NA, not available.

important consequences for health (Song, 2017). In addition to calcium, bone also contains cells, other inorganic mineral crystals and extracellular organic matrix, such as collagens. Bone structure can deteriorate as age, for example, cross-linking between the collagens is changed during aging. Collagens can also be damaged by accumulation of advanced glycation end-products, another general feature of the aging process. The most common aging-related bone disease is osteoporosis, which is also the most frequent degenerative disease. About 200 million people suffer from osteoporosis around the world, and the incidence is much higher than that of cancer, cardiovascular disease and other diseases that have been widely studied (Cooper et al., 1992). The risk of bone fractures increases significantly in patients with osteoporosis. Slight movements and even walking can lead to bone fractures, especially hip and vertebral fractures. Clinical studies show that about 1/4 of patients with hip fracture die within one year after fracture. The lifetime incidence of any osteoporotic fracture is estimated to be 40% to 50% in women and 13% to 22% in men (Friedman and Mendelson, 2014).

The components of bone are maintained in a balance to prevent osteoporotic fractures. Estrogen decline in aged women after menopause can lead to bone loss. In addition, aging-related bone loss reflects the confluence of comprehensive molecular and cellular processes. Bone strength depends on bone mass that is typically expressed as bone mineral density (BMD) and on bone quality that is a multifactorial entity including bone structural and material compositional properties. Bone material composition properties are monitored by parameters like mineral/matrix ratio, mineral maturity/crystallinity (MMC), nanoporosity, glycosaminoglycan (GAG) content, lipid content and pyridinoline content (Paschalis et al., 2016). Among these parameters, pyridinoline content shows the greatest deviation between healthy aging and postmenopausal osteoporosis.

Regulators in bone responses with aging

The majority of bone cells are mesenchymal origin: SSCs, which generate their downstream progenitors of bone, cartilage, fat and stromal tissue; chondrocytes, which are responsible growth plate and its subsequent remodeling; osteoblasts, which synthesize bone matrix and facilitate the mineralization process; osteocytes, which respond to load and regulate bone resorption and formation; stromal cells, which modulate HSCs or bone marrow niche function; and pericytes, which are blood microvessel cells and regulate bone marrow fibrosis (Armulik et al., 2011; Chan et al., 2015; Comazzetto et al., 2021; Serowoky et al., 2020; Wang et al., 2022b). Except for mesenchymal origin cells, bone cells also include ECs, which are associated with osteoprogenitors, and osteoclasts, which are hematopoietic origin and they are multinucleated giant cells responsible for bone resorption following signals from osteoblasts and osteocytes (Boskey and Coleman, 2010; Tuckermann and Adams, 2021).

All bone cells have comprehensive interactions in the aging process. In a model of postmenopausal osteoporosis, the specific antibody against Semaphorin 4D (SEMA4D) that is secreted by osteoclasts and mediates communication between osteoclasts and osteoblasts effectively stimulates osteoblastic bone formation (Negishi-Koga et al., 2011). Mature osteoblasts are found to secrete WNT4 to inhibit Nuclear factor of kappa light polypeptide gene enhancer in B cells 1 (NFKB1) signaling in macrophages and osteoclasts and to provide protection against bone loss in ovariectomy (OVX) and aging mice (Yu et al., 2014). Except for WNT4 from mature osteoblasts, it is reported that preosteoclasts secrete Platelet derived growth factor B polypeptide (PDGFB) to induce $CD31^{hi}EMCN^{hi}$ (type H) vessel formation, both of which are significantly lower in OVX-induced osteoporotic mouse model (Xie et al., 2014). In the aged mice, the abundance of type H vessels and associated osteoprogenitors is strongly reduced and the administration of deferoxamine mesylate (DFM) leads to increased numbers of type H ECs and bone formation (Kusumbe et al., 2014). Furthermore, osteoblast-endothelial cell crosstalk via Slit guidance ligand 3 (SLIT3) which is as effective as parathyroid hormone to reverse the bone loss effects of OVX (Xu et al., 2018). One of the hallmarks of aging in mice and humans is the development of fatty marrow. Recent study identified a new stem cell population with adipogenic and osteogenic potential in marrow which decreases during aging. In addition, Diprotin A and sitagliptin, as well as Dipeptidylpeptidase 4 (DPP4) inhibitors, can improve bone regeneration (Ambrosi et al., 2017). Moreover, Peroxisome proliferative activated receptor gamma coactivator 1 alpha (PPARGC1A) regulates marrow SSCs lineage allocation and inhibit marrow adipogenesis and bone loss in the aging skeleton (Yu et al., 2018a).

Taking the lowest cell abundance into account, it has therefore been more difficult to understand molecular and cellular processes of skeletal stem/progenitor cells (SSPCs) with aging than other bone cells. The advances in research technology, such as fluorescence activated cell separation (FACS) and single-cell RNA sequencing identified multiple types of SSPCs originating from different skeletal sites, such as bone marrow, growth plate or periosteum (Ambrosi et al., 2019). While SSPCs from different sites have similar features with respect to cell surface markers, they are not identical, for example in differentiation capacity (Ambrosi et al., 2019). Recent studies have identified a skeletal stem/progenitor cell in human (hSSPC), and found that loss of Sirtuin1 (SIRT1) expression but reactivation by trans-resveratrol or a small molecule compound restores the differentiation potential of aged hSSCs *in vitro* (Ambrosi et al.,

2020; Chan et al., 2018). In the elderly, a progressive accumulation of senescent cells leads to elevated levels of proinflammatory mediator, a process known as "inflammaging". A low-grade anti-inflammatory drug can reverse a functional aging-associated decline of SSPCs (Josephson et al., 2019). Moreover, immune cells, including neutrophils and macrophages, secrete Grancalcin (GCA) to drive aging-related bone degeneration (Li et al., 2021b). In addition to aged environment affected by immune cells, aged SSPCs have been found to secrete Colony stimulating factor 1 (CSF1) to promote the formation of osteoclasts and generate an inflammatory degenerative niche (Ambrosi et al., 2021).

Except for dependence on cell-extrinsic factors, some aspects of aging are clearly rooted in cell-intrinsic alterations, such as genomic instability, epigenetic alterations et al. All normal bone cells have a limited lifespan, which is controlled by the genomic stability and TL. Alterations in the methylation of DNA or acetylation and methylation of histones, such as loss of H3K9me and H3K27me3, can induce epigenetic changes that contribute to aging process. Recent study has found that KDM4B, a H3K9me3 demethylase, whose ablation impairs SSPCs self-renewal and promotes stem cell exhaustion by inducing SAHF formation (Deng et al., 2021). Thus, as summarized in the Figure 13, there are different mechanisms of cell-extrinsic factors among bone cells as they age, which indicates potentially important therapeutic targets in the treatment of osteoporosis.

Interventions on osteoporosis

In people with osteoporosis, osteoblastic bone formation does not keep pace with osteoclastic bone resorption and leads to bone loss. Inhibition of osteoclastic activity with antiresorptive drugs such as bisphosphonates and denosumab (a monoclonal antibody against the osteoclast-inducing Receptor activator of nuclear factor kappa B ligand (RANKL)) have been the primary therapy for osteoporosis for many decades. However, these agents induce a low turnover state with decreased bone formation and increased bone brittleness. Now, it is an attractive option that blocking senescent bone cells secretome to lower bone resorption and promote anabolic effect on bone. For example, using INK-AATAC transgenic mice, which harbor a "suicide" transgene that enables AP20187-induced selective killing of cells expressing *cyclin dependent kinase inhibitor 2A* (*cdkn2a*), a marker gene of senescent cells, a previous study has shown that removing senescent cells, or blocking their secretory phenotype, results in a reduction in aging-related bone loss in mice (Farr et al., 2017).

Nowadays stimulation of bone formation without "frozen bone" would be an ideal goal for osteoporosis treatment, yet the only currently approved anabolic agent is teriparatide, a

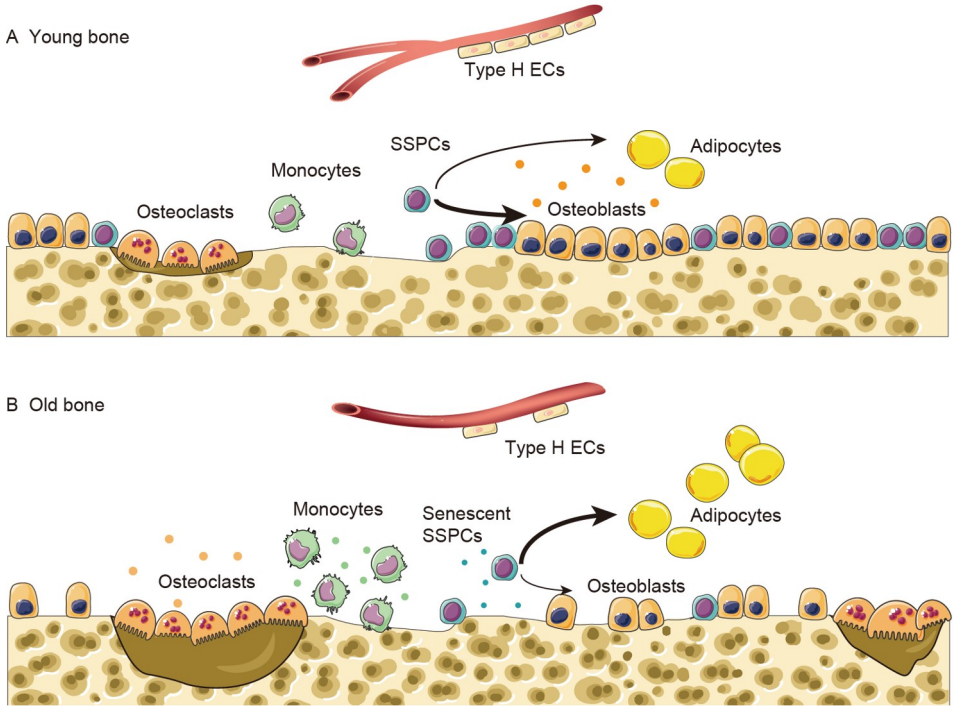

Figure 13 Schematic illustration of the cellular mechanism leading to bone aging. Mechanism of cell-extrinsic factors among bone cells in young and old bone. A, In the young bone, the cell populations involved in the bone remolding process contain SSPCs, osteoblasts, adipocytes, type H vessel endothelial cells (type H ECs), monocytes and osteoclasts. Osteoblasts secrete SLIT3 and PDGFB to instruct the microvasculature. B, In the old bone, osteoclasts secrete SEMA4D and inhibit osteoblast differentiation, monocytes secrete GCA to repress osteogenesis and promote adipogenesis and SSPCs secrete CSF1 to promote bone resorption.

recombinant parathyroid hormone (rPTH). However, several factors seem to limit the effectiveness of rPTH, such as a plateau of the net anabolic effect after 18–24 months treatment, the progressive decrease in responsiveness secondary to tachyphylaxis, and a depletion of the pool of mesenchymal osteoblast precursors (Drake et al., 2011). Thus, novel osteoanabolic strategies are highly desired to treat osteoporotic bone loss and stem cell therapy has emerged as a potent option.

Aging bone is also associated with reduced blood flow in human and animal models. As the same in the OVX-induced osteoporosis model, normal aging results in a profound diminishment of type H vessels and associated osteoprogenitors in the femur. Treatment of old mice with DFM can enhance HIF activity, increase type H vasculature and mineralize trabecular bone (Kusumbe et al., 2014). These experiments indicate that targeting blood vessels might be a promising therapeutic option for the treatment of osteoporosis either alone or in combination with anabolic or anti-resorptive drugs.

Readily available bone marrow and adipose tissue have used as a source for isolation and transplantation of bone forming cell populations. Considering of inflammaging in bone, anti-inflammatory drugs, like nonsteroidal anti-inflammatory drugs (NSAIDs), can be taken prior to elective orthopedic surgery to improve stem cell number (Josephson et al., 2019). Furthermore, another therapeutic strategy has been tested to reverse SSPC aging. Researchers mixed antibody molecules that specifically bind and block the function of CSF1 with Bone morphogenetic protein 2 (BMP2) that induce bone formation into a gel and placed the mixture at bone fracture site in aged mice to return bone regeneration to youthful levels (Ambrosi et al., 2021).

Last but not least, bone, as a supporting organ, has been challenged to resist mechanical signals. Conversely, sedentary lifestyles, combined with aging, have led to bone degeneration. Postmenopausal women and elderly men are encouraged to incorporate exercise into their daily regimen to improve bone strength (Hinton et al., 2015; Nilsson et al., 2017). *In vitro* studies performed on cells collected from the bones of aging women have shown that anabolic responses can be upregulated if the signals are dynamic. Physical exercise regimen can be used as a new strategy to improve bone quality while decreasing fat mass.

Summary and speculations

Bone provides mechanical support and regulates calcium ion homeostasis. The properties of bone change with age, in some cases, the function improves, but in others, function deteriorates. A new understanding of the regulators controlling aging bone cells, such as the discoveries of cell-extrinsic and cell-intrinsic factors, is pinpointing the pathways that can be targeted to reverse these age-dependent changes. Furthermore, prospective and integrative large-scale omics profiling, especially at the single-cell level, are discovering clinically actionable conditions, as well as novel molecular pathways for bone aging-related disease treatment. Considering organ-specific function of bone, combination of physical exercise and traditional 'medicine of disease' might become a perfect intervention on bone health.

Aging of skeletal muscle

Skeletal muscle accounts for over 40% of body weight. Its aging and the progressive decline of muscle mass and function during aging lead to dysregulation on mobility, breathing, vision, thermo-homeostasis, metabolism homeostasis, and immune-regulation (Englund et al., 2021; Yang and Hu, 2018). It plays a critical role in determining healthspan and lifespan. The aging of skeletal muscle has been considered a powerful driver for the organism aging.

Sarcopenia and skeletal muscle aging

The age-related progressive and generalized skeletal muscle disorder marked by the accelerated loss of muscle mass and functions, and the increased adverse outcomes including falls, frailty, and mortality has been termed as sarcopenia, which was recognized as an independent disease with an International Classification of Diseases-10 code in 2016 (Anker et al., 2016; Cruz-Jentoft et al., 2019). Sarcopenia has become a pandemic in old population. The causes of sarcopenia are not yet clear. Disuse, inadequate nutrients, reduction of sex hormone production, alteration of metabolism status, inflammation, endocrine changes, and factors originated from autocrine and paracrine all contribute to sarcopenia (Cruz-Jentoft et al., 2019; Gustafsson and Ulfhake, 2021). However, the molecular mechanism remains to be elusive. Furthermore, there is also no efficient treatment for sarcopenia thus far.

Molecular mechanism of aging-induced myofiber atrophy

The myofibers are the major cell type residing in skeletal muscle. Upon aging, the myofiber size and the contractile ability decline, which leads to the overall loss of muscle mass and function. Skeletal muscle contains two types of myofibers: the highly oxidative fast twitching myofibers and the mainly glycolysis slow twitching myofibers. The fast twitching myofibers are more sensitive to aging and tend to decay faster during aging (Anderson and Neufer, 2006), partly due to the conversion of fast twitching muscle fibers to the slow twitching muscle fibers with age, though the mechanism is not clear yet. Aging-induced muscle atrophy has been shown to be due to the loss of the balance between anabolism and catabolism. The decreased anabolism and increased catabolism in aged skeletal muscle together lead to the degradation of myofibril proteins and the decline of

muscle mass (Fealy et al., 2021). The elevated catabolism is due to the induced expression of muscle specific E3 ubiquitin ligase TRIM63 (MuRF1) and F-box protein 32 (FBXO32) with advanced age (Bodine and Baehr, 2014; Gumucio and Mendias, 2013). Transcription factor FOXO1 and FOXO3 have been shown to bind and activate the transcription of *Trim63* and *Fbxo32* (Glass, 2010b; Yin et al., 2018). The transcription activation of *Trim63* and *Fbxo32* by FOXO proteins is triggered by many stimuli. For example, the decreased insulin signaling can trigger the turn nuclear translocation of FOXO proteins to activate *Trim63* and *Fbxo32* transcription in starvation-induced muscle atrophy (Glass, 2010a). In contrast, a different signaling cascade has been applied in age-associated muscle atrophy. In aged muscle, increased nuclear localized CTNB1 (β-catenin) helps recruiting FOXO3 to the promoter of *Trim63* and *Fbxo32* to activate their transcription (Yin et al., 2018).

How the aging specific cell intrinsic signaling is turned on remains to be one of the major questions to be answered. The change of sex hormone levels has been attributed to the occurrence of age-dependent muscle atrophy (Geraci et al., 2021). Other muscle secreted factors (myokines) are also critical for the onset of muscle atrophy upon aging. The expression levels of Apelin, Irisin, BAIBA, BMP-7, Decorin, IGF-1, IL-15, SDF-1, Sesn2, SPARC, and VEGF-A decrease with age (Fuchs and Blau, 2020; Kwon et al., 2020). Apelin is a myokine secreted by the contracting skeletal muscle. It activates AMPK signaling to increase insulin sensitivity of skeletal muscle and promote glucose uptake (Dray et al., 2008). The concentration of plasma Apelin increases in response to aerobic exercise (Kwon et al., 2020). Sesn2 is another myokine producing exercise effects to improve muscle metabolism by activating Akt signaling (Martyn and Kaneki, 2020). The decreased expression of these exercise effective myokines with age partially explains the beneficial effects of exercise for sarcopenia and whole-body homeostasis.

The expression levels of DKK3, IL-6, and MSTN increased with age (Crescioli, 2020; Yin et al., 2018). DKK3 is a member of Dickkporf protein family. It is secreted by the aged skeletal muscle and induces the nuclear translocation of β-catenin to switch on FoxO3-dependent *Fbxo32* and *Murf1* transcription, therefore inducing muscle atrophy (Yin et al., 2018). Reducing DKK3 level by siRNA in old muscle can rejuvenate skeletal muscle (Yin et al., 2018). It is a potential target for sarcopenia drug development.

MSTN is a TGF-β family member secreted by skeletal muscle. *Mstn* knockout mice displayed excessive muscle growth, suggesting that the increased amount of MSTN during aging may contribute to the loss of muscle mass (Gutierrez-Salmean et al., 2014; McPherron et al., 1997). The mechanism of aging-induced muscle loss remains to be explored, which will uncover more targets for the development of new medicines treating sarcopenia.

The aging of skeletal muscle stem cells and the decline of regeneration ability

Upon aging, the regeneration ability of skeletal muscle also declines dramatically. Skeletal muscle has high regeneration ability due to the existence of a group of tissue stem cells named MuSCs which reside between basal lamina and myofiber plasma membrane (Fu et al., 2015a). Reduced MuSC number has been reported in aged skeletal muscle and is partly attribute to the declined muscle regeneration ability (Brack et al., 2007; Chakkalakal et al., 2012). However, the impaired MuSC activation, proliferation, and differentiation abilities have been shown to contribute more to the loss of regeneration ability in skeletal muscle.

The cellular intrinsic signaling pathways alter in aged MuSCs. In old MuSCs, p38/MAPK signaling is abnormally activated, which leads to impaired asymmetric division and self-renewal of MuSCs by activating cell cycle inhibitor $p16^{INK4}$ (Sousa-Victor et al., 2014; Sousa-Victor et al., 2022). p38/MAPK inhibitor treatment restores the asymmetric division and self-renewal ability of MuSCs (Bernet et al., 2014; Cosgrove et al., 2014). Consistently, reducing the level of $p16^{INK4}$ in old MuSCs restores the self-renewal capability (Cosgrove et al., 2014). Notch signaling is down-regulated in aged MuSCs, which results in MuSCs spontaneous differentiation, cell death due to mitochondrial catastrophe of MuSCs, and depletion of MuSCs from the stem cell pool (Bjornson et al., 2012; Liu et al., 2018a; Mourikis et al., 2012). The disruption of Notch signaling may attribute to the reduced number of MuSCs in old skeletal muscle.

With age advancing, DNA mutation also accumulates. It has been reported that 13 mutations per genome per life-year are found in human MuSCs (Franco et al., 2018). The accumulation of DNA mutations also contributes to the loss of function of MuSCs in old skeletal muscles. MuSCs in advanced age show increased level of DNA damage represented by phosphorylated H2AX, which may be due to the accumulated DNA damage upon aging (Sinha et al., 2014). The epigenetic landscape changes in aged MuSCs. DNA methylation directly regulates the expression of key myogenic genes (Wang et al., 2021a). Accumulation of stochastic DNA methylation changes and the increased DNA methylation heterogeneity in promoter regions disrupt the coherently knitted transcription network in old MuSCs (Hernando-Herraez et al., 2019). The repressive chromatin modification H3K27me3 also increases in aged MuSCs (Liu et al., 2013), suggesting that some important functional genes are repressed. The AMPK/$p27^{kip1}$ signaling is reduced in aged MuSCs, leading to declined autophagy and accumulation of damaged proteins and organelles (White et al., 2018). The expression of SIRT1 decreases in aged MuSCs leading to metabolic switch from OXPHOS to

glycolysis and premature differentiation (Ryall et al., 2015; Zhang et al., 2016). Consistent with the metabolic switch, the cellular level of NAD^+ decreases (Ryall et al., 2015; Zhang et al., 2016). Furthermore, the mitochondria unfolded protein response (UPR^{mt}) also declines and causes the accumulation of unfolded proteins and mitochondria damages (Fealy et al., 2021). However, not the expression of all genes downregulated in aged MuSCs. The expression of Hoxa9, a homeobox transcription factor, is activated in aged MuSCs (Schwörer et al., 2016). Wnt, JAK/STAT, and TGF-β signaling pathways are also upregulated in MuSCs with advancing age (Hong et al., 2022). In addition to the dysregulation of transcription, post transcriptional regulatory network is also disrupted in aged MuSCs. The expression level of RNA binding protein Msi2 decreases, which causes the de-repression of microRNA7a-1 (miR7a-1) maturation, and the accumulated miR7a-1 in turn leads to MuSC differentiation defects in old MuSCs (Yang et al., 2022). The dysregulation of overall gene expression in aged MuSCs attributes to the loss of functions in aged MuSCs.

Besides the cell intrinsic changes in aged MuSCs, the microenvironment of MuSCs also has many changes with age advancement. The old muscle fibers secrete reduced amount of granulocyte colony stimulating factor (G-CSF), which reduces the frequency of asymmetric cell division and $Pax7^{Hi}$ MuSC subpopulation (Li et al., 2019a). The old myofibers secretes increased amount of FGF2, which causes the premature activation of MuSCs and exhaustion of MuSCs (Chakkalakal et al., 2012). Consistently, the expression of downstream component of FGF2 signaling-β1 Integrin also decreases, further impairing the FGF2 signaling in old MuSCs (Rozo et al., 2016). Another ECM molecule fibronectin also shows reduced expression, which leads to impaired MuSC attachment to the niche and exhaustion of MuSCs in old muscles (Lukjanenko et al., 2016). Other cell types in the old microenvironment of MuSCs also regulate the activity of MuSCs. The Fibro-adipose progenitors (FAPs) are a group of cells present in the mesenchyme of skeletal muscles. FAPs secret Wnt1-inducible signaling pathway protein 1 (WISP1) to promote MuSCs proliferation. Old FAPs produce less WISP1, which reduces the proliferation of MuSCs (Lukjanenko et al., 2019). Immune cells are important microenvironment components of MuSCs. Balanced amount of TNFα, IFNγ, IL-1α, and IL-13, produced by immune cells are required to promote MuSCs proliferation (Fu et al., 2015b). In old mice, immune cells have altered responses and delayed phase transition to muscle injury. The altered inflammation process delays muscle regeneration. In old muscle, increased TNFα level and the disrupted balance of cytokines reduce the proliferation of MuSCs and increase fibrosis after muscle injury (Wang et al., 2018c). Changes of the levels of other microenvironment factors such as NFκB, IL-33, and CCL-2 also cause defects in MuSCs proliferation and differentiation leading to the declined regenerative ability after muscle injury during aging (Blanc et al., 2020; Kuswanto et al., 2016; Oh et al., 2016).

Summary

The aging of skeletal muscle mainly affects two categories of the skeletal muscle functions. On the one side, the homeostasis of protein synthesis and degradation is disrupted in terminally differentiated myofibers leading to increased protein degradation and decreased myofiber size. On the other hand, the aged skeletal MuSCs display declined activity that leads to skeletal muscle regeneration defects. The aging of skeletal muscle causes the exhaustion of the existing myofibers and the disruption of the regeneration mechanism, which together lead to the loss of skeletal muscle functions in aged population. The loss of DNA integrity, chromatin structure, mitochondria homeostasis, hormone balance, and ECM component stability are all attributed to the aging-related muscle function loss (Figure 14). The study of the mechanism governing the aging of skeletal muscle and MuSCs has only been initiated. Many more extensive investigations are urgently needed to provide more targets for the development of interventions for sarcopenia to restore muscle functions and to improve the regenerative ability of MuSCs. Approaches targeting MuSCs, microenvironments, metabolic molecules, and the endocrine functions of skeletal muscles have all been under heated investigation to achieve the goal of skeletal muscle rejuvenation.

Skin aging

The skin is the outermost organ of the human body, which undertakes a variety of important functions such as protection, temperature regulation, feeling, immunity, and social interaction. As the main organ that constitutes the appearance of the human body, the aging of the skin and its appendages is also the most intuitive manifestation of human aging. Here, we comprehensively explore the aging characteristics of the skin and its possible mechanisms from the clinical manifestations and molecular mechanisms.

Structure of skin

The skin is composed of skin epithelium, its underneath dermis and hypodermis (Figure 15). The skin epithelium is a keratinocyte derived tissue that contains interfollicular epidermis (IFE), sweat gland (SWG), and hair follicle (HF) with sebaceous gland (SG) attached. It is one of the few human tissues that self-renew constantly in normal homeostasis and self-repair after wounding. This ability attributes to its lifelong reservoirs of stem cells (SCs) in both IFE and HF (Fuchs, 2008; Fuchs and Blau, 2020). The IFE is a stratified tissue composed of terminally differentiated stratum cor-

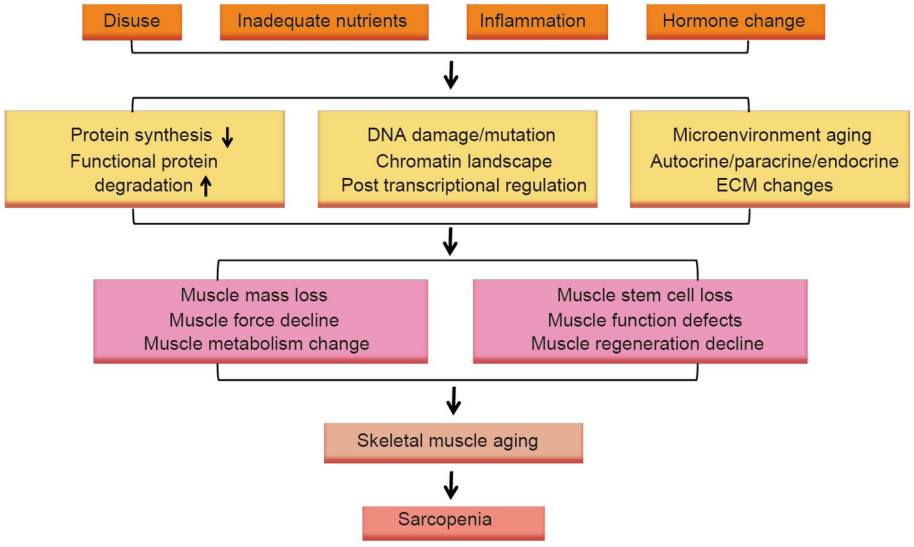

Figure 14 Main mechanism of skeletal muscle aging. During the process of aging, physiological changes such as disuse, inadequate nutrients, elevated inflammation level, decline of sex hormones, and alternation of other hormones function together to repress protein synthesis and induce the expression of muscle specific E3 ligases Atrogin 1 and MuRF1 degrading functional proteins. The disruption of the protein homeostasis attributes to the muscle mass, muscle force, and the metabolic function loss. On the other hand, DNA damage and mutations accumulate in MuSCs during aging. The chromatin landscape, transcription and post transcription network change significantly in aged MuSCs. The microenvironment cell types and numbers change during aging. Meanwhile the alteration of autocrine/paracrine factors and ECM components also occur during aging. These factors together lead to reduced MuSCs number, functions, and eventually regenerative ability of old skeletal muscles. The loss of muscle mass, force, and regenerative ability are representative features of skeletal muscle aging, which leads to sarcopenia.

neum, intermediate granular & spinous layers, and a proliferative basal layer that harbors IFE SCs (Fuchs, 2008). The HF is a mini-organ that undergoes cyclic regeneration (anagen), degeneration (catagen) and quiescence (telogen) phases, known as the hair cycle. This is driven by cyclic activation of HF stem cells (HFSCs) that are enriched in a bulge region of HF outer root sheath (ORS) underneath SG (Blanpain and Fuchs, 2006). HFSCs and IFE SCs are normally confined to their own lineage during homeostasis, but can show significant lineage infidelity under wounding and disease conditions (Ge et al., 2017). The dermis contains an abundance of dermal fibroblasts, which are responsible for the synthesis and secretion of dermal ECM composed of collagen, elastic fibers, fibronectin, elastin, and other related proteins (Haydont et al., 2019). Human skin dermis has two major parts: an upper papillary layer with loose ECM and high cell density, and a deeper reticular layer with dense ECM and low cell density (Watt and Fujiwara, 2011). In contrast to the highly dynamic skin epithelia, dermal fibroblasts are surprisingly static during normal homeostasis. Localized cell loss in the dermis is found to be compensated by membrane extension rather than division or migration of neighboring cells (Marsh et al., 2018).

Clinical characteristics of skin aging

Skin aging phenotypes in human include those induced by physiological age (intrinsic aging) and those induced by genetic or environmental factors. Physiological skin aging symptoms typically include pale, dry, and wrinkles in epidermis, as well as reduced wound healing rate, alopecia and hair graying. At histological level, aged skin is characterized by atrophy of epidermis, atrophy of dermis structures, decrease of fibroblasts, and miniaturization of HF (Levine, 2020; Matsumura et al., 2016). Photoaging is a common form of environmental factor-induced skin aging. It accounts for many age-associated changes in sun exposed skin areas. Chronic exposure to ultraviolet (UV) irradiation from sunlight is considered to be a major cause of photoaging, which differs from physiological skin aging in several aspects. These include dry and deep-wrinkled skin, yellow discoloration and pebbly surface of the skin, hyperpigmentation, solar lingerie and actinic keratoses. Histologically, the dermis displays accumulated aberrant elastin fibers, glycosaminoglycans as well as disorganized fibrillin and tropoelastin. Increased number of hyperplastic fibroblasts and inflammatory cells are also observed in photodamaged skin. In addition to UV irradiation, ionizing radiation (IR) can also induce aging-like chronic skin symptoms, including dermal/epidermal atrophy, dermal hypovascularization, alopecia, severely impaired wound healing capability, and vulnerability to infection (Ryan, 2012). Premature skin aging phenotypes are also observed in certain genetic disorders. Progeria, or HGPS, patients develop marked alopecia, wrinkle, and irregular epidermal pigmentation (DeBusk, 1972). Dyskeratosis congenita (DC) is also associated with abnormal skin pigmentation, premature hair graying /loss, and other noncutaneous abnormalities (Kirwan and Dokal, 2008).

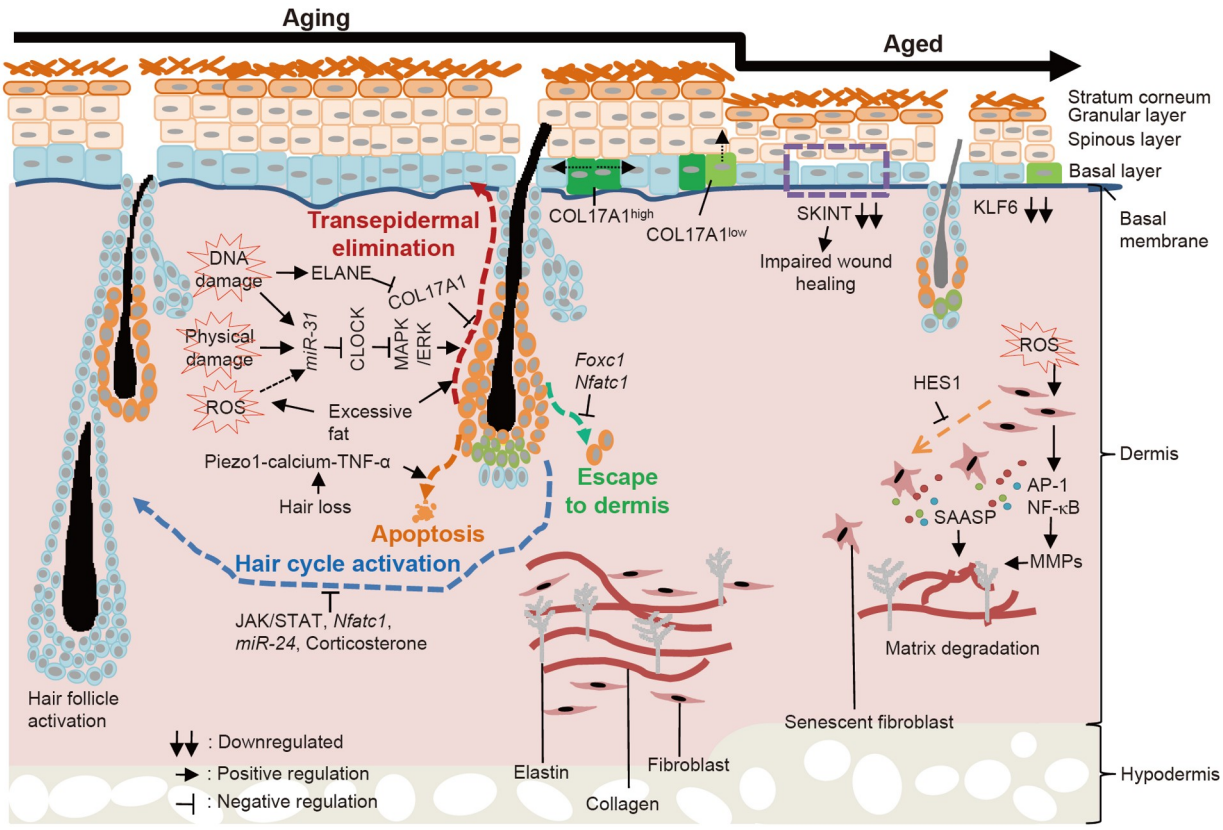

Figure 15 Schematic of skin aging characteristics and mechanisms. Left and right parts represent aging and aged skin respectively as indicated in the top diagram. Orange pebbles: HFSC; green pebbles: hair germ; dashed arrows: cell fate transitions. Multiple mechanisms, including stem cell exhaustion, transcriptional deregulation, extracellular matrix degradation and etc., have been found to be involved in the skin aging process that leads reduced epidermal/dermal thickness, HF miniaturization, and impaired wound healing in aged skin.

The aging of dermis

In aged skin, the dermis displays reduced overall thickness and reduced tissue density in both papillary and reticular layers. The aged dermis also shows alteration in dermal ECM composition/organization and degradation of the dermal collagen network and elastin fibers, which play an important role in skin elasticity, leading to wrinkles, laxity and sagging (Haydont et al., 2019). Increased ROS is believed to play a major role in dermal aging. It can stimulate the expression of matrix metalloproteinases (MMPs), which degrade collagen and elastin. This is likely related to ROS-induced activation of activator protein 1 (AP-1) and nuclear factor kappa B (NF-κB) pathways (Chiang et al., 2013). Cellular senescence plays a prominent role in dermal fibroblast aging. It is found that the number of senescent fibroblasts grows exponentially in skin of aging primates (Herbig et al., 2006) and senescence-related $p16^{INK4a}$ (CDKN2A)-positive cells increase in dermis during aging of human (Zou et al., 2021). Dermal fibroblasts from intrinsically aged skin also show increased production of skin aging-associated secreted proteins (SAASPs) that overlap with classic SASPs in matrix degradation and proinflammatory aspects (Waldera Lupa et al., 2015). Transcriptional analysis shows that aged human dermal fibroblasts have elevated ECM disassembly genes and reduced cell proliferative genes. This is in agreement with the defective dermal collagen network and increased cellular senescence in aged dermis (Zou et al., 2021).

The aging of epidermis

Aged human epidermis (IFE) is characterized by reduced overall thickness, diminished rete ridges, and atrophy of spinous and basal layers (Zou et al., 2021). Similar to human, aged mice also have a thinner epidermis, with flattened structure and reduced number of epidermal stem cells, which may attribute to stem cell competition based on differential hemidesmosome components COL17A1expression (Liu et al., 2019b). Unlike dermis, the role of cellular senescence in IFE aging is relatively obscure. Accumulation of $p16^{INK4a}$ positive cells in aged human IFE has been observed (Ressler et al., 2006; Zou et al., 2021). However, the $p16^{INK4a}$ positive cells in IFE are found to be mostly melanocytes but not keratinocytes (Victorelli et al., 2019). It is conceivable that in the highly dynamic IFE tissue, senescent keratinocytes would be quickly cleared by cellular competition. That said, increased expression of senescence-related genes is detected

in aged human IFE (Zou et al., 2021). It has also been shown that keratinocytes from aged human IFE are more prone to cellular senescence in 3D culture in comparison with their young counterparts (Nassour et al., 2016). Another feature of aged epidermis is its impaired ability to initiate immune response, making the skin more susceptible to infections and inefficient at wound healing. This is related to the impaired ability of aged epidermal keratinocyte to activate epithelial-immune crosstalk genes (Keyes et al., 2016). However, activation of inflammatory genes is also observed in aged human epidermis (Zou et al., 2021). Thus, whether it is a cause or consequence of epidermal aging remains to be determined (Ge et al., 2017).

The aging of hair follicles

Hair graying and hair thinning/loss are prominent human aging phenotypes. They are linked with stepwise miniaturization of hair follicles and progressive loss of HFSCs in both mouse and human (Matsumura et al., 2016). One mechanism for the aging-induced HFSC depletion is transepidermal elimination. HFSC is normally confined to the HF lineage but can commit trans-differentiation into epidermis upon wounding (Ge et al., 2017). It was found that DNA damage can cause proteolysis of COL17A1 in aging HFSCs and lead to HFSC transepidermal elimination (Matsumura et al., 2016). A recent study showed that physical and/or genotoxic damages cause *miR-31* upregulation in aging HFSCs. The upregulated *miR-31* down-regulates *Clock* to activate mitogen-activated protein kinases/extracellular signal-regulated kinase (MAPK/ERK) and drives HFSC transepidermal elimination. Interestingly, prominent miR-31 upregulation is also observed in aged human epidermis, hinting its role in IFE aging as well (Dries et al., 2021). Notably, blocking the HFSC transepidermal elimination by COL17A1 overexpression, *miR-31* ablation or MAPK/ERK inhibition can significantly slow down HF aging in mouse, indicating that HF aging is not just wear and tear, but a programmed and targetable process (Dries et al., 2021; Matsumura et al., 2016). Interestingly, obesity can also induce HFSC transepidermal elimination by generating excess ROS, leading to accelerated hair thinning (Morinaga et al., 2021).

Several other mechanisms may also contribute to the HFSC depletion during aging. By *in vivo* imaging of aged mouse skin, HFSCs are observed to escape from their niche and directly migrate into dermis. This phenomenon may attribute to dampened expression of FOXC1 and NFATC1 in the aged HFSCs (Zhang et al., 2021b). Loss or miniaturization of hair shaft often proceeds HF miniaturization during aging. A recent study showed that hair shaft miniaturization in aging and genetic hypotrichosis disorders can cause HFSCs depletion via apoptotic loss mediated by Piezo1 activation (Xie et al., 2022b).

It should be noted that while HF miniaturization and HFSC depletion represent the terminal fate of HF aging, they may not be the only mechanisms underlying HF aging. Many prevalent HF aging phenotypes, such as baldness and hair graying, can develop at much earlier time points. Increased HF dormancy is a typical sign in early stages of HF aging. In aged mouse skin, even the HFs without visible miniaturization are becoming increasingly dormant due to impaired proliferation capability of their HFSCs. This is related to the intrinsic upregulation of a pro-quiescence gene *Nfatc1* in the HFSCs and cannot be efficiently rescued by parabiosis with young animals (Keyes et al., 2013), suggesting that it is an intrinsic feature of aged HFSCs. Another study showed that the proliferation of aged mouse HFSCs is inhibited by age-associated inflammation via Janus tyrosine Kinase/signal transducer and activator of transcription (JAK/STAT) signaling, whose blockade can reactivate aged HFs from their dormancy (Doles et al., 2012). JAK/STAT inhibitors have been developed into a new regimen to treat alopecia and have shown promising results in clinical trials (Zheng and Tosti, 2021). However, it is still controversial whether such an immediate HF activation regimen is beneficial to delay or reverse HF aging in the long-term. In mouse, marked HFSC depletion is observed following JAK inhibitor-induced hair regeneration (Doles et al., 2012). In JAK inhibitor treated alopecia patients, significant disease recurrence was also observed (Zheng and Tosti, 2021). Recently, it was shown that stress hormone corticosterone can suppress HFSC activation and that removal of such inhibition enhances HF regeneration without discernible defects in the long-term (Choi et al., 2021). Another study showed that *miR-24* limits the intrinsic sensitivity of HF progenitors to growth stimuli and that removal of such limitation also enhances HF regeneration without discernible side effects (Miller et al., 2021). These studies have enlightened promising new avenues to alleviate HF dormancy.

Pigment changes during aging

Hair graying is another phenotype that can arise at early stage of HF aging. Hair graying is usually a progressive process starting from middle age. Color of a single hair can sometimes swing between black and white (Rosenberg et al., 2021), suggesting that it is a reversible process at least in early stages. Aging-related hair graying largely attributes to loss of melanocytes and melanocyte stem cells (MeSCs) (Nishimura et al., 2005). This can be resulted from the aging of HFSCs that forms the niche environment of MeSCs (Lu et al., 2020b). It was also found that stress-induced neuronal activity can cause hair graying by norepinephrine mediated MeSCs loss (Rosenberg et al., 2021; Zhang et al., 2020a). Notably, human hair graying is not only a color change but also associated with alterations in the biophysical properties and growth rate of the hair shaft (Van Neste and Tobin, 2004), implicating profound changes in the graying HF's

epithelial lineage.

Summary

Skin aging is a long-term and complex process, and so far, we have only uncovered the tip of the iceberg. As can be seen from the above research, skin aging is not a simple wear and tear process, nor is it just a cumulative process of senescent cells. It is more like a systematic evolutionary process that is regulated by a specific mechanism and can be targeted for intervention at specific nodes. As more and more in-depth research progresses, ageless appearance may not be an untouchable dream.

Aging and the reproductive system

Aging is characterized by the progressive deterioration of multiple organ functions and degenerative diseases (Hernandez-Segura et al., 2018). For example, the decline in reproductive ability is one aspect of aging. The reproductive system is a collection of organs that generates both gametes and sex hormones. By maintaining endocrine homeostasis, it plays a crucial role in promoting the birth of healthy offspring and coordinating physiological functions. The risk of infertility increases when a female partner is over 35 years old (Ahmed et al., 2020). In addition, aging-related menopause in women is accompanied by an endocrine disorder and is associated with an increased risk of several major health complications, including osteoporosis, cardiovascular diseases, recurrent depression, and others (Secomandi et al., 2022). Male fertility also declines with age, but it is more gradual and is accompanied by endocrine homeostasis disorders such as late-onset hypogonadism (LOH). Symptoms of LOH include a loss of libido, erectile dysfunction, and decreases in muscle mass and bone density. Moreover, benign prostatic hyperplasia (BPH) and prostate cancer (PCa) are typical age-related diseases impacting the male reproductive system and affecting the quality of life in elders (Kaufman et al., 2019). Since reproductive health is strongly linked to the overall health of organisms, reproductive aging leads to various age-associated disorders in both women and men. Here, we summarize the latest developments in our understanding of the mechanisms and possible treatments that support healthy female and male reproductive aging (Table 2).

Aging of the female reproductive system

The female reproductive system mainly comprises the ovaries, fallopian tubes, uterus, and vagina. Ovarian aging is considered to be the most crucial contributor to female reproductive aging, primarily due to the progressive decrease of the quantity and the quality of the oocytes. The number of oocytes is finite at birth and decreases throughout a woman's lifetime. The age-related decline in oocyte quality is centered on a declining ability to re-initiate meiosis, fertilization (Brighton et al., 2017), and poorer preimplantation outcomes (Secomandi et al., 2022). Ovarian aging leads to increased menstrual cycle variability and eventual menopause onset. The menopausal transition has been clinically and endocrinologically evaluated according to the standardized classification defining "stages of reproductive aging" (2011 STRAW+10). Accordingly, decreases in antral follicle count (AFC) detected through ultrasound is direct evidence of the gradual loss of the ovarian reserve. In addition, decreases in the anti-Müllerian hormone (AMH), inhibin-B, and estradiol concentrations, along with elevated follicle stimulating hormone (FSH) levels, indicate that the oocyte pool and quality gradually diminish (Duncan et al., 2018).

The molecular mechanisms underlying age-associated

Table 2 The underlying mechanisms of female and male reproductive system aging

Gender	Organs	Mechanisms	References
Female	Ovary	DNA damage	(Li et al., 2021a; Ruth et al., 2021; Turan and Oktay, 2020)
		Chromosome mis-segregation and aneuploidy formation	(Duncan et al., 2018; Nakagawa and FitzHarris, 2017; Turan and Oktay, 2020)
		Telomere shortening	(Kalmbach et al., 2013; Park et al., 2021)
		Improper epigenetic modifications	(Li et al., 2021a; Liu et al., 2021c)
		Mitochondrial dysfunction	(Chiang et al., 2020; Wang et al., 2020e)
		Elevated inflammatory response	(Lliberos et al., 2020; Pertynska-Marczewska and Diamanti-Kandarakis, 2017)
	Uterine	Elevated inflammatory response	(Cavalcante et al., 2020)
		Increased secretion of SASP factors	(Brighton et al., 2017; Shirasuna and Iwata, 2017)
Male	Testis	Epigenetic changes	(Yatsenko and Turek, 2018; Zambrano et al., 2021)
		Inappropriate redox balance	(Beattie et al., 2015; Weir and Robaire, 2007)
		Decreased UPR-related proteins	(Cavalcante et al., 2020; Zhao et al., 2019)
		Apoptosis	(Akbari et al., 2022; Zhao et al., 2019)

decline in oocyte quality and quantity is multifactorial and is poorly understood. One of the most central determinants of oocyte quality is chromosome segregation during meiosis. Age-related failure of accurate chromosome segregation typically leads to oocyte aneuploidy, which can be as high as 30%–60% as is much higher than aneuploidy rates in sperm (~1%–2%) (Duncan et al., 2018). Chromosome cohesion in oocytes degenerates naturally with age, leading to erroneous kinetochore-microtubule (K-MT) attachments and multipolar spindles with unstable microtubules (Nakagawa and FitzHarris, 2017). In addition, compromised spindle assembly checkpoints in the presence of DNA damage leads to a higher frequency of chromosome mis-segregation, premature chromatid separation, and aneuploidy (Turan and Oktay, 2020). Recently, 290 genetic determinants of ovarian aging were identified among 200,000 women of natural menopausal age, indicating a wide range of DDR-related genes (Ruth et al., 2021). Progressive telomere shortening results in age-related oocyte dysfunction, manifested by genomic instability and apoptosis (Kalmbach et al., 2013). Inappropriate epigenetic modifications, including abnormal DNA methylation, histone modifications, and non-coding RNA-regulated modifications, can also contribute to oocyte aging (Li et al., 2021c). For example, deficiency of Tet1, a major DNA demethylase, leads to premature ovarian failure (Liu et al., 2021c). Mitochondrial dysfunction also affects ovarian aging, including mtDNA mutations, disrupted ROS neutralization, and defective disposal of dysfunctional mitochondria (mitophagy) (Chiang et al., 2020). Consistently, single-cell transcriptomic analysis in cynomolgus monkey ovaries identifies dysregulation of antioxidative pathways in aged oocytes (Wang et al., 2020e). In addition, oocytes are surrounded by GCs, which are essential for oocyte growth and developmental competence. Accumulating evidence demonstrates that age-related GC dysfunction contributes to decreased oocyte quality. Excessive ROS production also causes the downregulation of FSH-receptor expression in GCs, resulting in an adverse response to FSH and subsequent impaired follicle development with age (Martín-Ramírez et al., 2021). Furthermore, advanced glycation end products (AGEs) lead to protein damage and increases in inflammatory reactions, eventually inducing ovarian function decline (Lliberos et al., 2020; Pertynska-Marczewska and Diamanti-Kandarakis, 2017).

The rapid decline in reproductive outcomes with age can also be attributed to uterus aging and is related to the development of uterine dilatation and uterine fibrosis in the muscular layer and stroma (Cavalcante et al., 2020). Uterine fibrosis is a consequence of chronic collagen deposition, which may be caused by long-term periodic exposure of uterus to estrogen. Estrogen activates the PI3K/AKT/mTOR signaling pathway and upregulates profibrotic microRNAs targeting a group of genes, including genes that encode ovarian steroid receptors, inflammatory factors, and TGF-β and its receptors (Brighton et al., 2017). Additionally, cellular senescence induces abnormalities in decidualization of endometrial stromal cells and a defective endometrial response, resulting in improper cross-talk between the endometrium and fetus, followed by implantation abnormalities (Brighton et al., 2017).

To date, there are no clinically-feasible techniques to reverse age-related ovarian and uterine dysfunction. However, senotherapy has recently emerged as a promising intervention. For example, senolytics, such as dasatinib and quercetin, not only reduce the accumulation of ROS in the ovary, but also have an antifibrotic effect on the uterus (Wang et al., 2018a). Melatonin, a strong antioxidant, can increase telomere length and reduce inflammation during ovarian aging (Secomandi et al., 2022). Calorie restriction can also prevent aging-associated oocyte aneuploidy (Mishina et al., 2021). In addition, developments in mitochondrial replacement therapy, nuclear genome transfer, and autonomous germline mitochondrial energy transfer (AUGMENT) techniques improve oocyte quality (Reddy et al., 2015; Woods and Tilly, 2015), however clinical safety considerations require further evaluation (Tvrdá et al., 2021).

Aging of the male reproductive system

The main organs of male reproductive system include the testis, epididymis, vas deferens, prostate gland, and seminal vesicle. Unlike in woman, male reproductive system aging is only mild to moderate with age. The main function of the testis is to produce sperm and androgen. Age-associated testis dysfunction often leads to a decline in sperm quantity and quality, which is one of the major contributors to male infertility (Tvrdá et al., 2021). Age-related changes in testis include the narrowing of tubular diameter, thinning of the seminiferous epithelium, thickening of the basal membrane, and increased fibrosis. Another prominent manifestation of testicular aging is the mosaic-like lesions that develop in the seminiferous tubules, ranging from spermatogenesis to complete sclerosis of the seminiferous epithelium (Perheentupa and Huhtaniemi, 2009). In addition, a reduction in the number of Sertoli cells, Leydig cells, and spermatogenic cells, as well as vacuolization of the Sertoli cells and multinucleation of the spermatogenic cells are observed in aged testis (Perheentupa and Huhtaniemi, 2009). More importantly, a slight decrease in overall testosterone levels, a more pronounced decrease in free testosterone, an increase in FSH, and a moderate increase in LH may indicate impaired testicular function among older men (Kaufman et al., 2019).

Paternal aging leads to lowered genetic quality of sperm production and is characterized by increases in DNA damage and fragmentation, mutations, and aneuploidies (Yatsenko and Turek, 2018). Moreover, various epigenetic changes, including aberrant DNA methylation, are another major

factor that contributes to lowered sperm quality (Yatsenko and Turek, 2018; Zambrano et al., 2021). The antioxidant enzymatic activities of GPX1, GPX4, and SOD in aged sperm are diminished, resulting in redox imbalance (Weir and Robaire, 2007). Additionally, abnormal UPR is viewed as the primary contributor to testicular aging. The expression of UPR-related proteins, such as GRP78, p-PERK, p-EIF2α, p-IRE1α, ATF6α, and XBP1, decreases progressively in the testis throughout aging (Zhao et al., 2019), whereas the expression of proteins involved in ER stress-related pro-apoptosis (e.g., CHOP, Caspase 12, and p-JNK) and mitochondrial apoptosis (e.g., cytochrome c, Caspase 9, and Caspase 3) is greater in the aged testis (Zhao et al., 2019).

Several studies have indicated that common oral antioxidants (e.g., vitamin C, vitamin E, vitamin D, selenium, folic acid, zinc, and carnitine) can improve sperm quality (i.e., sperm quantity, morphology, and motility) (Tvrdá et al., 2021). Vitamin D supplementation reportedly alleviates symptoms associated with male reproductive aging (Jeremy et al., 2019). Melatonin modulates endocrine activities in Leydig cells and influences the growth, proliferation, and energy metabolism of Sertoli cells, consequently promoting spermatogenesis (Matzkin et al., 2021). Moreover, senolytics and senomorphics such as quercetin or resveratrol can reduce testicular lipid peroxidation and increase antioxidant enzyme activities (Catalase, GSH reductase) (Hamza et al., 2021). In addition to these pharmacological treatments, testosterone replacement therapy is a common clinical intervention.

Age-related changes to the hypothalamic-pituitary-gonadal axis

The hypothalamic-pituitary-gonadal axis (HPG axis) system is composed of the hypothalamus, pituitary gland, and gonadal glands, which regulate endocrine function and maintain the stability of the reproductive system. Parvocellular neurosecretory cells in the hypothalamus release gonadotropin-releasing hormones (GnRH) into the pituitary portal system, followed by follicle-stimulating hormone (FSH) and luteinizing hormone (LH) that are released by endocrine cells in the anterior pituitary gland and circulate throughout the body, acting either on the ovaries in women or the testes in men. Estrogen and testosterone are then released into the blood. These two hormones act on cells expressing the corresponding receptors, turning on the expression of specific genes or sending feedback signals to the superior organs of this axis (Vadakkadath Meethal and Atwood, 2005).

In women, the most important function of the HPG axis is to regulate periodic changes in the ovary and endometrium. FSH and LH can promote follicular maturation and ovulation (McGee and Hsueh, 2000). In men, LH stimulates Leydig cells to produce testosterone and FSH stimulates Sertoli cells to produce androgen-binding proteins. Sertoli cells bind to testosterone, which is necessary for normal sperm production (Araujo and Wittert, 2011). HPG axis activity decreases overall with age, and has a greater impact on women. Compared to young women, older women exhibit a lower amplitude of the LH surge prior to ovulation, the peak appears later, and the balance between stimulating and inhibitory effects associated with GnRH neuron hormone secretion is disrupted (Downs and Wise, 2009). During the menopausal transition period, serum FSH levels increase and menstrual cycles become irregular until menopause, when women are no longer considered fertile (McTavish et al., 2007). Perimenopausal women are also at risk of depression and cognitive decline. In this case, estrogen supplementation may serve both preventive and protective functions (Greendale et al., 2009).

For males, HPG axis activity decreases more slowly over time and is consistent with the gradual decline of fertility. For instance, GnRH secretion in the hypothalamus decreases, LH secretion pulse frequency increases and the amplitude decreases, testosterone production decreases, and the negative feedback sensitivity mediated by testosterone decreases with age (Veldhuis et al., 2009). Aged men with abnormal HPG axis activity experience a variety of symptoms, including the loss of libido, physical weakness, and depression. As such, androgen replacement therapy may be beneficial (Veldhuis et al., 2009).

Perspectives

With increasing age, reproductive functions decline in both women and men, leading to decreases in fertility. We currently lack an in-depth, comprehensive understanding of reproductive system aging and its mechanisms, therefore effective therapeutic strategies are limited and urgently needed. The emergence of new technologies such as single-cell sequencing, including scRNA-seq and single-cell transposase-accessible chromatin sequencing (scATAC-seq), together with spatial transcriptomics, enable us to establish a comprehensive landscape of reproductive system aging, and uncover key contributors. Additionally, the development of higher resolution microscopy imaging techniques will afford future study of cellular and molecular mechanisms with greater accuracy. Collectively, these cutting-edge technologies will provide opportunities for a more thorough understanding of reproductive aging mechanisms, paving the way for the development of clinical intervention to mitigate reproductive aging and related diseases.

Aging intestine and microbiome

Aging-associated change of the gastrointestinal tract and the gut microbiome has been increasingly implicated in human health and diseases, and has become a focal point in biomedical studies. The aging process is often accompanied with gut microbiota dysbiosis and disturbed intestinal func-

tion including intestinal epithelial stem cells (IESCs) impairment, thinner mucus, increasing intestinal permeability, and chronic inflammation (Branca et al., 2019; DeJong et al., 2020). The shifts in both the host intestine and gut microbes are tightly interacted and linked to a range of age-related diseases including metabolic disorders and neurodegenerative diseases.

Aging intestine

IESCs are multipotent cells located at the base of the intestinal crypt and are responsible for the renewal of epithelial cells and the maintenance of intestinal homeostasis (Branca et al., 2019). Elevated proliferation and mis-differentiation of IESCs are observed in aged *Drosophila* intestine, along with excessive expression of ROS and ROS-related pathways like c-Jun N-terminal Kinase (JNK) signaling pathway that may contribute to homeostasis breakdown (Branca et al., 2019; Jasper, 2020). Further studies on a mammalian aging model confirm the dysfunction of IESCs including diminished and less complicated organoids, higher level of apoptosis and weaker intra-crypt movement with age (Branca et al., 2019). The reduced expression of Wnt pathway genes in the crypt may account for the age-related phenotypes because promoting *Wnt3a* expression restores the function in mouse and human organoids (Jasper, 2020).

Another aspect of aging intestine is the injured intestinal barrier characterized by thinner mucus layer, increasing intestinal permeability and chronic proinflammatory state (Branca et al., 2019; DeJong et al., 2020) (Figure 16). The mucus secreted by goblet cells protects the epithelium from the pathogens. Studies have revealed that the change of mucus with aging process varies in different locations. In the gastric and duodenal layers, the thickness of mucus layer is not changed, while the ileum of aged mice displays a slightly thicker layer (Branca et al., 2019). In contrast, the colonic mucosal barrier tends to be thinner and defective in aged mice, with an abnormal O-glycosylation of mucin mediated by over-expression of *miR-124-3P* (Branca et al., 2019; Huang et al., 2020). In addition, increased intestinal permeability is observed in many studies, yet the mechanisms are not fully elucidated (Branca et al., 2019; DeJong et al., 2020). One possible mechanism is tight junctions (TJs) dysfunction. TJs are big junctional complexes attaching intestinal epithelial cells, which play a critical role in blocking microbes and macro-molecules (Branca et al., 2019). Altered expression of TJ proteins like tight junction protein 1 (TJP1), claudin 2, and F11 receptor (F11R) are observed in aged human or primates (Branca et al., 2019). The aging-associated chronic proinflammatory state—characterized as "inflammaging" by consistently elevated local and systemic levels of inflammatory mediators such as interleukin 6 (IL6) and tumor necrosis factor (TNF), might be a driver of "the leaky gut" as uplifted inflammatory cytokines induce the TJ remodeling via myosin light chain kinase (MYLK) pathway and suppress the expression of TJ proteins (Branca et al., 2019; DeJong et al., 2020). Conversely, increased intestinal permeability allows opportunities for pathogens and their products like lipopolysaccharide (LPS) to go into the circulatory system and aggravates the inflammaging state (Bosco and Noti, 2021; DeJong et al., 2020; Ferrucci and Fabbri, 2018).

Aging microbiome

The aging intestine shapes the gut microbiota. Studies have shown that the alpha diversity of gut microbiota is negatively correlated with frailty, a physiologically susceptible state prevalent in elders (O'Toole and Jeffery, 2015). Compared with younger healthy individuals, the loss of diversity is related to the dominance of *Bacteroides*, and an enrichment of *Alistipes*, *Eubacteriaceae* and pathobionts like *Streptococcus* and *Enterobacteriaceae*, accordingly the depletion of *Bifidobacteriaceae*, *Ruminococcaceae and Faecalibacterium prausnitzii* (DeJong et al., 2020; Wilmanski et al., 2021; Zhang et al., 2021h). The decreased taxa are recognized as short-chain fatty acids (SCFAs) producers. SCFAs have been reported to be important energy sources to epithelial cells and gut microbes, thus promoting intestinal integrity, biodiversity and alleviating inflammation, and are negatively correlated with the development of chronic diseases like diabetes and neurodegenerative diseases (DeJong et al., 2020; Martin-Gallausiaux et al., 2021; Vijay and Valdes, 2022; Zhu et al., 2021b). Therefore, the depletion of SCFA-producing species may play a role in the frailty and age-related diseases. Recent studies have demonstrated that supplementation of *Bifidobacterium adolescentis* can ameliorate osteoporosis and neurodegeneration of premature aging mice, and further improve healthspan and lifespan in *Drosophila* and *C. elegans* (Chen et al., 2021b). On the other hand, the mucin-consuming bacteria *Akkermansia* is confirmed on its role in maintaining the barrier function and metabolic homeostasis (DeJong et al., 2020; Shin et al., 2019). Although its abundance either decreases (Shin et al., 2019) or increases with age (Zhang et al., 2021h) in different cohorts, it is shown in multiple studies that gavage of some strains contributes to the mitigation of age-related loss of mucin, chronic inflammation and extension of lifespan of progeroid mice (Bosco and Noti, 2021; DeJong et al., 2020).

Focal studies on the centenarians, who display less susceptibility to chronic diseases have different gut microbial patterns with frailer elders (Kong et al., 2019). Gut microbes in centenarians tend to be more diverse and unique, with a less dominance of core abundant taxa (e.g., *Bacteroides* and *Faecalibacterium*) and increased prevalence of rare taxa (e.g., *Methanbacteriaceae*) (DeJong et al., 2020; O'Toole and Jeffery, 2015; Wilmanski et al., 2021). Potentially beneficial taxa including *Akkermansia*, *Bifidobacterium*, *Clos-*

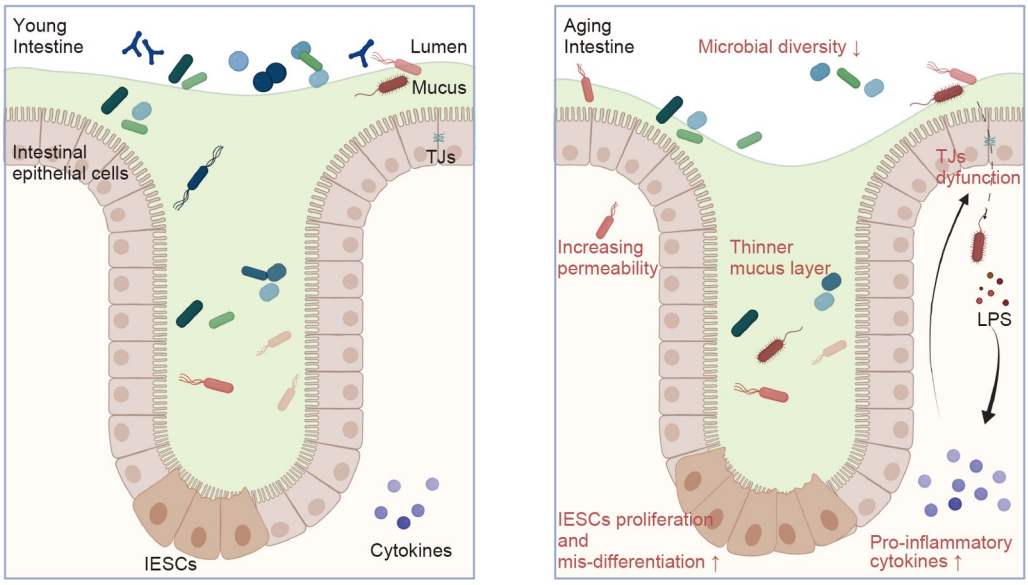

Figure 16 Aging intestine and microbiota. Adapted from "Keystone Gut Microbiota Species Provide Colonization Resistance to invading Bacteria", by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates. Overview of aging intestine and microbiota changes. Left: young intestines are characterized by normal epithelial functions of the intestine, no inflammations and relatively high diversity of microbiome. Right: aged intestines have thinner mucus layer, lower microbial diversity, and higher degree of leakage due to tight junctions (TJs) dysfunction, resulting in inflammation by LPS and other microbial products.

tridium XIVs and *Christensenellaceae* are enriched, indicating the potential role as microbial signatures of longevity (Biagi et al., 2016; Ferrucci and Fabbri, 2018). These taxa have been documented to be beneficial in promoting immune function, counteracting the obesity effects, and maintaining metabolic homeostasis (Biagi et al., 2016). However, it is unclear the extent of biological age's impact on microbial compositions, as studies in these long-lived cohorts have also demonstrated that centenarians tend to live in healthy lifestyle, especially a high-fiber diet favored by those taxa (DeJong et al., 2020). In addition, centenarians are likely to have unique metabolic profiles. Metagenomics analysis of long-living subjects (age >99 years) reveals a prevalence of xenobiotic degradation and a rearrangement in nutrient metabolism (Rampelli et al., 2020). Moreover, a large-scale Japanese cohort study has demonstrated that novel bile acid biosynthetic pathways are enriched in centenarians, the products of which exert antimicrobial functions and consequently improve intestinal homeostasis (Sato et al., 2021).

Gut microbiota and aging-related diseases

An increasing collection of studies has reported that gut dysbiosis is related to age-related diseases including metabolic disorders, including type 2 diabetes (T2D), cardiovascular diseases (CVD), neurodegenerative diseases (AD and Parkinson's disease (PD) (Vijay and Valdes, 2022; Zhu et al., 2021b). A general trend in gut microbiome of those diseases is that they share similar pattern with aging microbiota, either in composition or metabolome (Ferrucci and Fabbri, 2018; Vijay and Valdes, 2022). For instance, one study on mild cognitive impairment has shown that the patients display higher prevalence of *Bacteroides*, just like frail elders (Saji et al., 2019). A lack of SCFA-producing bacteria and the inflammaging state, as previously suggested, are shown to be responsible for a higher risk of metabolic and neurodegenerative diseases. Mechanistically, SCFAs can activate pancreatic beta cells through two receptors, or induce intestinal L-cells to secrete glucagon-like peptide 1 (GLP-1), and subsequently stimulate the insulin release indirectly (Priyadarshini et al., 2016; Vijay and Valdes, 2022).

In addition to SCFAs, disturbed intestinal barrier and high endotoxemia (systemic inflammation) triggered by excessive LPS invasion can be causative effects to the onset of T2D (Salamone et al., 2021), and even neurodegenerative diseases (Zhu et al., 2021b). Inflammation may induce the misfold of synuclein alpha aggregates, the primary neuropathological markers of PD, which are abundant in the enteric nervous system and subsequently detectable in the brain with the pathogenesis (Wang et al., 2020c; Zhu et al., 2021b). Another study has shown that LPS can access the hippocampus and cause cognitive impairments like memory disturbances (Saji et al., 2019). In contrast, fecal microbiota transplantation (FMT) of young mice (3–4 months) to old mice (19–20 months) can save the seniors from aging-associated impairments in brain immunity and cognitive behavior (Boehme et al., 2021).

The relationship between generation of CVD and gut microbiota is even more complicated. It is suggested in a large cohort including 6,953 individuals that systolic blood pressure negatively correlates with the abundance of *Lactoba-*

cillus species, consisting with many investigations of improved heart function after the administration of *Lactobacillus*, in which the mechanism may be the activation of T helper 17 cells to improve hypertension (Battson et al., 2018). In addition, *Lactobacillus* gavage to aged rats results in attenuated hyperlipidemia via the promotion of 5′ adenosine monophosphate-activated protein kinase (AMPK) pathway (Lew et al., 2020). Apart from SCFAs modulating blood pressure, other important microbial metabolites are found including such as trimethylamine (TMA) and phenylacetic acid (Nemet et al., 2020; Vijay and Valdes, 2022). TMA is oxidized into trimethylamine N-oxide (TMAO) in the liver, which is well-documented as a putative mediator of CVD, though the mechanism has not been fully understood (Battson et al., 2018). In addition, phenylacetic acid can be transferred into phenylacetyl glutamine and enhances the platelet responsiveness and thrombosis potential through adrenergic receptors (Nemet et al., 2020).

Summary

The aging process alters gut physiology and microbiota. Aged gut is characterized by hyper-proliferation and dysfunction of IESCs, increasing intestinal permeability, thinner colonic mucus layer and elevated chronic inflammation. Affected by the intestinal state, the frailer—physiologically older gut microbiota tends to be less diverse and depleted of SCFA-producers, while in centenarians who have a healthier gut, the microbial composition is more diverse and individualized with unique metabolic profiles. The shifted gut microbiota modifies the host metabolism and immunity in feedback and can be a risk factor for age-associated diseases like type 2 diabetes, T2D, CVD and neurodegenerative diseases. Further researches may explore the role of gut microbes as the therapeutic targets to aging-related diseases and provide deeper understanding into microbiota-host interactions in aged people.

Aging and immune senescence

Aging is a complex physiological process accompanied by the development of aging-related diseases such as inflammatory and autoimmune diseases, cancers, infections, and neurodegenerative disorders (Calcinotto et al., 2019; Ma et al., 2021; Wang et al., 2021b; Zheng et al., 2020). One of the major pathogenic factors is immune senescence-induced dysfunction, which promotes the responses to self-antigens whereas weakens the body's resistance to foreign antigens and tumor cells, thereby increasing the risk of aging-related diseases (Calcinotto et al., 2019; Leng and Pawelec, 2022). This immune dysfunction due to increased age is defined as physiological immune senescence (PHIS). PHIS can also be defined as aging-associated immune senescence (AAIS), and is mainly triggered by DNA damage, loss of the telomere protective functions and the promoted expression of the *cdkn2a* locus which encodes P16 and ARF (Calcinotto et al., 2019; Kim and Sharpless, 2006). Apart from aging-related immune decline, pathological microenvironment also can induce immune cell senescence exhibiting similar phenotype to PHIS, which can be defined as pathological immune senescence (PAIS). Both PHIS and PAIS affect immune response and disease progression in pathological processes. Here, we mainly focus on the most recent findings that implicate the features and biological consequences of immune senescence in different disease contexts.

Inflammation and autoimmunity

Elderly individuals have an increased potential to develop chronic inflammation and autoimmune diseases, reflected by high levels of proinflammatory markers in blood and tissues along with other endogenous damage products such as ATP, uric acid or circulating DNA, which is a typical characteristic of PHIS and is defined as inflammaging (Calcinotto et al., 2019; Latz and Duewell, 2018). In addition, aging-induced proinflammatory microenvironment also promotes the secretion of a serial of cytokine products, including IL-1, TNF, IL-6, IL-8, IL-13, IL-18, IFNα, IFNβ and TGF-β, which is also known as SASP. The SASP in turn functions in a paracrine manner, facilitating PAIS of neighboring immune cells and thus disease progression (Latz and Duewell, 2018).

The NLRP3 inflammasome, an intracellular multiple-protein complex mainly located in the innate immune cells like macrophages, is actively involved in inflammaging during physiological aging process. It is widely known that the abovementioned SASP directly activates NF-κB via cognate receptors, and further induces inflammasome assembly in innate immune cells (Latz and Duewell, 2018). Apart from that, the exogenous and endogenous stimuli, including stress-induced mitochondrial ROS and metabolites, can also promote the assembly and activation of the NLRP3 inflammasome (Latz and Duewell, 2018; Zhou et al., 2011). Reciprocally, recent studies have demonstrated that the NLRP3 inflammasome is a major regulator of age-related diseases, including type 2 diabetes and metabolic disorders, through IL-1β and IL-18 production (Latz and Duewell, 2018). In addition to inflammaging, it has been reported that NLRP3 inflammasome also mediates aging-related adipose B cells accumulation and inhibits lipolysis through IL-1R signaling, impairing metabolic homeostasis in aged visceral adipose tissue (Camell et al., 2019).

As mentioned above, the DNA damage in physiological aging or pathological contexts is emerging as a key mediator of immune senescence (Muñoz-Espín and Serrano, 2014), which also induces the accumulation of large amount of free DNA. As one of the most important cytoplasmic DNA sensors, cGAS is mainly expressed in innate immune cells to mediate inflammatory responses through inducing the acti-

vation of IRF3, MAPK and NF-κB signaling (Miller et al., 2021). It has been reported that the cytoplasmic cGAS-mediated DNA sensing pathway promotes senescence and SASP in both human and mouse cells, aggravating tissue inflammation upon ionizing radiation. Therefore, assuring chromatin homeostasis, DNA repair, eliminating cytoplasmic DNA, as well as inhibiting cGAS-mediated downstream signaling activation, can act to prevent excessive activation of innate immune signaling in the aging and pathological context (Miller et al., 2021).

Neutrophil, a critical component of innate immunity, have been shown to age rapidly and exhibit enhanced proinflammatory activity compared with neutrophils newly produced from the bone marrow. The aged neutrophils are characterized by progressive loss of L-selectin (CD62L) expression, but upregulated expression of CXCR4 (Bordon, 2015). Recent studies have demonstrated that toll-like receptor (TLR)2 and TLR4 signaling are critical for neutrophil aging (Bordon, 2015; Zhang et al., 2015a), and further analysis indicates that microbiota is involved in neutrophil aging and thus causes remote organ damage through TLR downstream MYD88 signaling pathways. Clinically, depleting the microbiota can evidently reduce aged neutrophils *in vivo* and thus improve inflammation-related organ injury (Zhang et al., 2015a).

Dendritic cells (DCs), the most professional antigen presenting innate immune cells, function as a bridge to connect innate and adaptive immunity. Although there is no obvious difference on the number and phenotype of DC subsets between young and aged subjects, the functions of DCs are compromised with age, reflected by impaired phagocytosis, antigen presenting and migration activity (Agrawal and Gupta, 2011). In addition, aged DCs become insensitive and tolerant to the stimulation of foreign antigens, while their response to self-antigens is greatly enhanced (Agrawal and Gupta, 2011), which may be the reason for the increased risk of autoimmune and infectious diseases in elderly people.

Previous studies have proven that T cells undergo major changes in aged individuals, and T cell senescence reversely regulates multiple organismal features, including metabolic, cognitive, physical, cardiovascular alterations, and even the lifespan (Desdín-Micó et al., 2020). Except for hyperactivation, the senescent T cells also exhibit decreased T cell receptor diversity, SASP, short telomeres, signs of DNA damage, low expression of the costimulatory molecules CD27 and CD28, high expression of terminal-differentiation markers and elevated β-galactosidase activity (Liu et al., 2020a). Moreover, the $CD27^{-}CD28^{-}CD8^{+}$ senescent T cells also express a protein complex containing the agonistic NK receptor NKG2D and the NK adaptor molecule DAP12, which promotes the killing of NKG2D ligands-expressing cells (Pereira et al., 2020). In terms of cellular metabolism, T cell senescence is associated with increased activation of mTORC1 signaling, glycolysis and glutaminolysis, and limiting mTORC1 activity can be used to prevent T cell senescence-induced dysfunction (Suzuki et al., 2018).

In aged T cells, DNA is also accumulated in the cytoplasm due to mild DNA damage (Wang et al., 2021e). Different from innate immune cells, a recent study has demonstrated that T cells sense cytoplasmic DNA in a cGAS-independent manner. KU complex, a Ku70/80 heterodimer, is observed abundantly present in the cytoplasm, and recognizes aging-induced accumulated cytoplasmic DNA in $CD4^{+}$ T cells, leading to enhanced $CD4^{+}$ T cell-mediated autoimmunity. In this process, KU complex senses cytoplasmic DNA and recruits DNA-PKs, which then phosphorylate the downstream kinase ZAK and further activate AKT and mTOR pathways, and eventually promote $CD4^{+}$ T cell activation and proliferation, thereby potentiating the development of autoimmune inflammation in aged individuals. Interestingly, calorie restriction or targeting ZAK kinase through a selective inhibitor greatly suppresses DNA-boosted $CD4^{+}$ T cell activation and thus alleviates autoimmune inflammation in aged mice (Wang et al., 2021e).

Treg is a subset of $CD4^{+}$ T cells that maintains peripheral tolerance and prevents autoimmune diseases (Salminen, 2020). Increased proportion of Treg populations have been observed in multiple tissues during physiological aging process in both human and mouse (Elyahu et al., 2019; Salminen, 2020). However, Tregs are more prone to become senescent than conventional T cells, and Tregs from aged mice are less efficient in suppressing effector T cell proliferation and activation *in vitro*. The senescence of Treg induced by PHIS also promotes PAIS of other immune cells, together accelerating the development of inflammaging and autoimmunity (Guo et al., 2020; Salminen, 2020). Interestingly, a recent study suggests that targeting DCAF1/GSTP1/ROS axis can rejuvenate Treg function and related pathologies in aging individuals (Guo et al., 2020).

Except for senescent T cells, the age-associated B cell subsets have also been widely studied for many years. The aged B cells are mainly defined as $B220^{+}CD19^{+}$ splenic cells that lack CD21, CD23, CD95 and CD43 with decreased B cell receptor diversity, while CD11c is an important marker for B cell aging. Similar to Tregs, the percentage and number of splenic age-associated B cells increase along with age (Cancro, 2020). Functionally, the age-associated B cells are significantly associated with humoral autoimmune diseases, including scleroderma and rheumatoid arthritis. Mechanistically, TLR9 and TLR7 are critical mediators for age-associated B cell expansion in mediating the induction of autoimmunity (Cancro, 2020).

Anti-tumor immunity

Cancer is a common public health problem in old people, which is mainly due to accumulated gene mutation, in-

sensitivity of adaptive immunity and chronic inflammation (Calcinotto et al., 2019; Lian et al., 2020). However, the tumor development in aged mouse model is controversial, which probably depends on different tumor microenvironments and non-immune mechanisms (Fane and Weeraratna, 2020). In Balb/C mice, although the tumor growth derived from breast cancer cell line 4T1 is similar between young and aged mice, the response to immune checkpoint blockade (ICB) therapy is lower in aged mice due to the decreased IFN signaling and antigen presentation (Sceneay et al., 2019).

In addition to the inflammatory microenvironment, tumor also can trigger PAIS of infiltrated T cell probably through metabolic reprogramming, thereby inhibiting T cell survival and anti-tumor effector functions (Liu et al., 2018c). Recent studies have demonstrated that the PAIS induction of tumor-infiltrating T cells is attributed to the upregulation of group IVA phospholipase A_2 that is caused by unbalanced lipid metabolism (Liu et al., 2021d). Apart from the senescence markers that are indicated above, the senescent T cells in tumor also display defective killing abilities due to the loss of perforin and granzyme, and show suppressive features by inhibiting the proliferation and effector functions of other immune cells, which further promote tumor development and progression (Liu et al., 2020a). Therefore, preventing T cell senescence and rejuvenating effector T cells can be beneficial for cancer immunotherapy.

Neurodegeneration

It is well known that aging is associated with cognitive decline, including AD, spinal cord injury, stroke, and multiple sclerosis (Rawji et al., 2016; Wu and Zhou, 2022). However, the underlying relationship between central nervous system (CNS) immune senescence and age-associated cognitive decline have not been well defined.

During aging, the CNS infiltrating monocyte-derived macrophages show decreased proinflammatory cytokine secretion, phagocytosis, and chemotaxis (Rawji et al., 2016). Similarly, when stimulated with TLR agonists such as LPS *in vitro*, senescent macrophages show significantly decreased production of the proinflammatory cytokines when comparing with young macrophages (Rawji et al., 2016). Nonetheless, activated PGE2 signaling in aged myeloid cells can promote the sequestration of glucose into glycogen and metabolic reprogramming, which drive harmful proinflammatory responses and cognitive decline (Minhas et al., 2021). Therefore, it is controversial for the proinflammatory role of aged macrophage in aging brain.

In contrast, senescent CNS-resident microglia display a primed, more proinflammatory phenotype in aging brains (Harry, 2013). Mechanistically, there is a loss of inhibitory ligand-receptor interactions in aged microglia, and misfolded proteins, such as amyloid-β, accumulate in aging brain, which induces inflammatory cytokines in microglia (Rawji et al., 2016). In addition, it has been found that there is a striking buildup of lipid droplets in microglia with aging in mouse and human brains, which is defined as lipid-droplet-accumulating microglia (LDAM), and results in the production of ROS and secretion of proinflammatory cytokines (Marschallinger et al., 2020). In addition, impaired phagocytosis is also a characteristic of aged microglia associated with the accumulation of inhibitory myelin debris and amyloid-β, resulting in microglia-mediated neurological disease progression (Harry, 2013). Moreover, comparing with young microglia, the senescent microglia also display decreased motility and migration capacity in response to stress-induced injury (Rawji et al., 2016). Interestingly, as the transcriptomic profile analysis has demonstrated, the signatures of aged microglia show differences in cell adhesion, axonal guidance, cell surface receptor expression and actin assembly between human and mouse (Galatro et al., 2017). In the future, it needs to address the function of human-specific microglial senescence under physiological and neuropathological conditions.

Summary

Here, we summarize the main features and biological consequences of PHIS and PAIS for different immune cells under physiological aging and multiple pathological conditions, including inflammatory and autoimmune diseases, cancers, and neurodegenerative disorders (Figure 17). Accumulating evidence shows that either PHIS or PAIS leads to immune cell dysfunction and disease progression. However, the pathogenic function of immune senescence is not absolute, depending on the immune cell types and disease context. For example, the induction of Treg cell senescence can be used to improve cancer-immunotherapy, whereas it can also cause autoimmunity under inflammatory conditions. The challenges for future study are to understand the molecular mechanism that drives PHIS and PAIS, and the interplay between immune senescence and different disease context, which will provide a clearer picture of immune senescence in different pathologic conditions and more definite targeting strategies of clinical treatment for aging-related diseases.

Aging of hematopoietic stem cells

HSCs are able to generate all of the blood cells and themselves throughout lifespan, which are called differentiation and self-renew respectively. During aging, HSCs exhibit impaired regeneration and homing capacity, while the frequency is increased which is considered as the compensation of functional loss (Jaiswal and Ebert, 2019). Meanwhile, aged HSCs also display differentiation bias towards myeloid lineage and furthermore clonal hematopoiesis, which is a risk factor for a series of hematopoietic diseases, including ane-

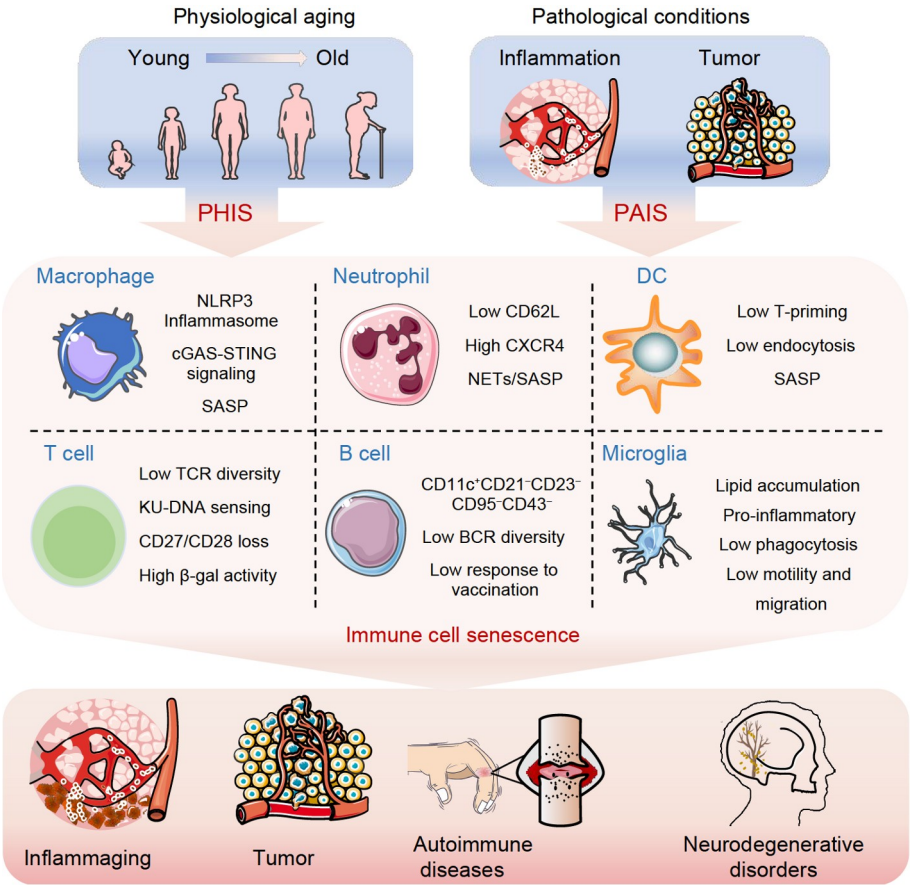

Figure 17 The inducers, features and consequences of immune senescence. Both physiological aging and pathological conditions can induce immune senescence, according to which it can be divided into PHIS and PAIS, which exhibit different senescence-related features in different innate and adaptive immune cells, including macrophages, neutrophils, DCs, T cells, B cells, and microglia. As a consequence, either PHIS or PAIS leads to immune cell dysfunction which in turn results in the development of aging-related diseases, including inflammaging, tumor, autoimmune diseases and neurodegenerative disorders.

mia, immune disorder, myelodysplastic syndromes and myeloid leukemia (Mejia-Ramirez and Florian, 2020). Aged HSCs are not sufficient to maintain the homeostasis of the immune system. Therefore, elucidating the molecular mechanisms behind HSC aging is important to identify potential therapeutic targets for restoring immune system, further reducing the incidence of inflammatory disorder and hematological diseases in the elderly. Throughout this section of the review, we focus on a series of phenotypic and functional alterations in murine studies to characterize aged HSCs (Figure 18).

Replication stress or DNA damage?

The nature of quiescence is a low metabolism state that preserves HSCs from replicative and oxidative stress. However, HSCs accumulate considerable DNA damage while exposed to endogenous and exogenous stress during aging. In response to DNA damage, active HSCs adopt efficient HR repair whereas quiescent HSCs are disposed to NHEJ repair, which is prone to introduce mutation and genome instability to impair HSCs (Mohrin et al., 2010). Specific DNA damage drives the growth advantage of hematopoietic cells or even blood malignancies under some conditions, which is called clonal hematopoiesis (Mandal et al., 2011). Although it has been reported that the accumulated DNA damage in aged HSCs is repaired when entering cell cycle, telomere shortening leads to DNA damage accumulation in quiescent HSCs and telomere-free chromosome ends are not easy to be repaired (Wang et al., 2014b). Alternative study has shown that DNA repair is equally efficient but the functional potential is weakened in aged HSCs compared with young ones, which indicates that replication stress but not DNA damage may account primarily for the declined function of aged HSCs (Flach et al., 2014). While, several studies have reported that HSCs rarely enter the cell cycle (Nakamura-Ishizu et al., 2014). How such a low frequency of DNA replication leads to DNA replication stress or accumulation of DNA damage is an important scientific question worthy of in-depth study.

ROS accumulation

A high level of oxidative metabolism provides aged HSCs

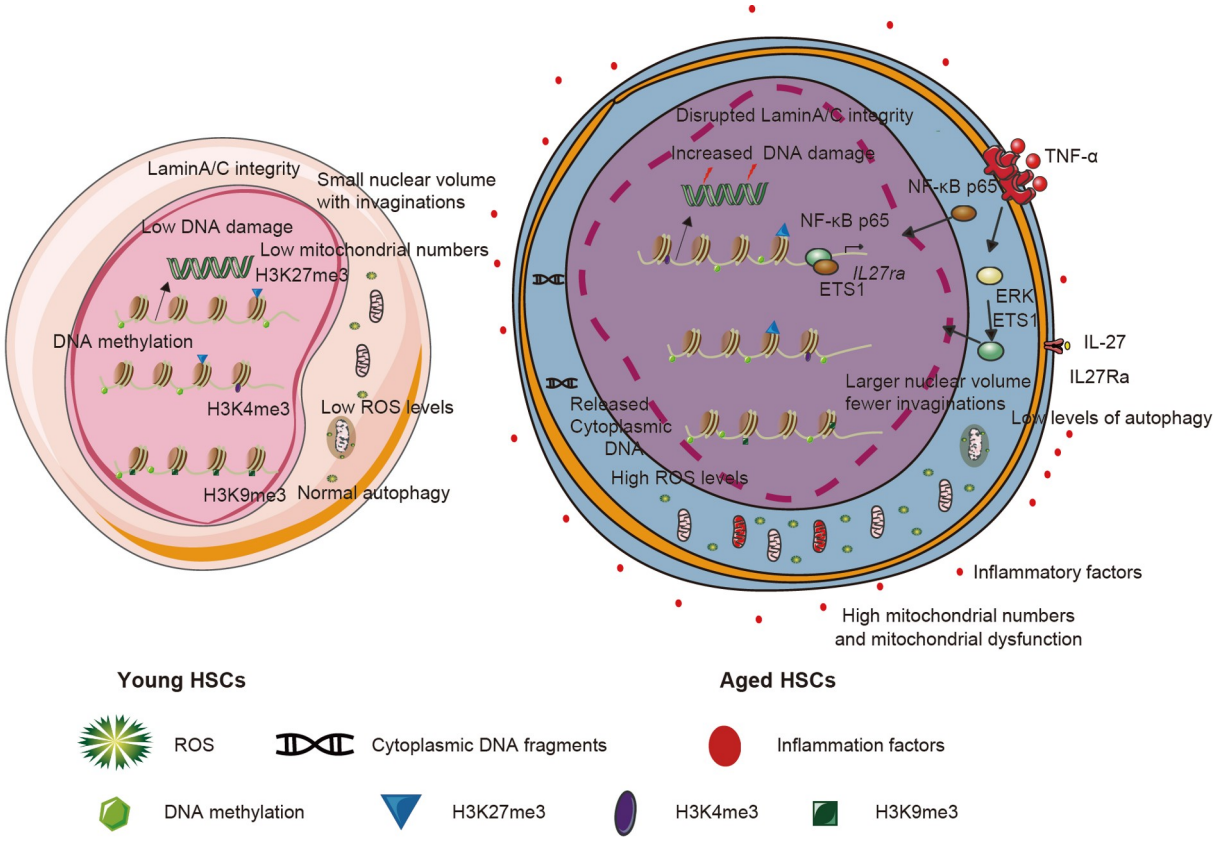

Figure 18 Hallmarks of HSC aging. The representative features of young HSCs and aged HSCs.

with both sufficient energy and toxic by-products ROS, and the latter leads to HSC exhaustion, which indicates that bioenergetically and biosynthetically balanced metabolism is critical to manage self-renewal capacity and quiescent status of HSCs (Ito and Suda, 2014). Once mitochondria suffer stresses, ROS production is remarkably increased and, in turn, promotes mitochondrial dysfunctions with DNA damage accumulation, which subsequently accelerates HSC aging (Nakamura-Ishizu et al., 2020). Elevated ROS oxidizes not only DNA, but also proteins and related cell signaling, which subsequently manipulates their function, stability, subcellular localization, mutual interactions and so on. Several previous studies have reported that dysfunction of FOXO3, ATM, TXNIP, MLL5 recapitulate the phenotype of aged HSCs by inducing ROS within them (Mattes et al., 2019). In line with this, the number of HSCs with low ROS levels decreases with age, while high ROS HSCs exhibit compromised proliferation, impaired self-renewal capacity, and further malignancy (Jang and Sharkis, 2007; Nakamura-Ishizu et al., 2020). Therefore, targeted scavenging of ROS or targeted manipulation of ROS-producing genes may rejuvenate HSCs.

Epigenetic modifications in aged HSCs

HSCs undergo both replicative stress and oxidative stress in the niche during aging, resulting in the erosive chromatin landscape, with DNA and histone modification alterations, and altered transcriptome. The changes of H4K16ac in aged HSCs are correlated with increased nuclear volume, fewer nuclear invaginations and chromosome 11 distribution (Grigoryan et al., 2018). Epigenetic modifications regulate the function of HSCs at different stages, and even slight epigenetic shifts reorganize HSC chromatin status and transcriptome. For instance, the switch of methyltransferase expression remodels heterochromatin in aged HSCs. Histone methyltransferase SUV39H1 is decreased, and as a result, heterochromatin modification H3K9me3 is globally reduced in aged HSCs (Shi et al., 2020a). Heterochromatin disarrangement increases chromatin accessibility and gene expression leakage, damaging differentiation potential and regeneration capacity of aged HSCs. Chromatin immunoprecipitation sequencing and DNA methylation between young and aged HSCs have shown that the number and coverage length of H3K4me3 peaks on HSC fingerprint genes and self-renewal genes are increased, while the coverage of H3K27me3 peaks on lymphoid differentiation genes is broadened. Additionally, the overall DNA methylation level increases in aged HSCs, and the binding sites for some transcription factors involved in HSC self-renewal program are hypomethylation, while the binding sites associated with

myeloid leukemia and differentiation are hypermethylated. Epigenetic modifications changes reorganize transcriptome with upregulation of HSCs self-renewal, inflammation, stress response, and leukemia-related genes, and down-regulation genes related to lymphoid differentiation, DNA repair, and chromatin silencing with HSCs aging (Sun et al., 2014).

HSCs niche

Adult HSCs mainly localize in bone marrow microenvironment, which is called "niche". HSCs niche is characterized as complex and dynamic network of interactions across a variety of cells, such as mesenchymal stromal cells, ECs, osteoblast and mature hematopoietic cells, which regulate HSCs self-renewal and differentiation (Morrison and Scadden, 2014). Several elegant studies have shown that HSCs niche modulates HSCs aging as cell-extrinsic mechanism. For example, young HSCs transplanted into aged recipients exhibit senescent phenotypes including increased myeloid differentiation, while aged HSCs into young recipients display reduced myeloid skewing but limited rejuvenation (Geiger et al., 2013). Niche cells are regulatory components of the niche *in vivo* that influence HSC function through different signaling pathways. The endosteum with low level of Notch1 signaling constrains HSCs close to the bone surface to maintain HSCs quiescence in young mice. However, aged HSCs are detached from endosteum, arterioles, nestin-GFP^{high} cells and megakaryocytes, which indicates that aging process may remodel HSCs niche (Zhang et al., 2020b). In addition to the direct interaction between niche cells and HSCs, niche (or systematic environment)-secreting factors may also impair HSCs function during aging process. Aging-increased inflammatory cytokines, such as IL-1, IL-6, TNF-α and IFN-γ, which are secreted by niche or other cells, promote the differentiation potential of HSCs towards myeloid/megakaryocytic lineages, and *vice versa*, aging-increased inflammatory cytokine-secreting cells release more inflammatory factors to accelerate HSCs exhaustion, forming a positive feedback loop (Kovtonyuk et al., 2016).

Inflammation

Aged HSCs exhibit differentiation skewing towards myeloid lineage over lymphoid cells, which is considered as one of the main driving factors of immunosenescence. Moreover, aging is also accompanied by increased systemic levels of multiple proinflammatory cytokines such as TNF-α and IL-1β, which is called chronic inflammaging. A growing body of works have revealed that inflammatory signals modulate HSCs proliferation, differentiation, self-renewal capacity and aging-related diseases. IFNs stimulate dormant HSCs to enter cell cycle, promoting HSC proliferation and eventually exhaustion (Sezaki et al., 2020). Interleukins are the key proinflammatory "emergency" signals, driving the myeloid differentiation of HSCs to recover myeloid cells rapidly during stress hematopoiesis (Ho and Méndez-Ferrer, 2020). The effects of TNF-α on HSCs are complex and ambiguous. Studies have shown that TNF-α restricts normal HSCs activity, and promotes HSCs aging through TNF-α→ERK→ETS1→IL27Ra pathway (He et al., 2020a). On the other hand, TNF-α promotes HSCs survival and myeloid differentiation by evading necroptosis under chronic inflammation (Yamashita and Passegué, 2019). These seemingly contradictory results may suggest a dosage-dependent effect of TNF-α on HSC function or different effect of TNF-α on different types of HSCs. An *in vivo* experimental assay has proven that aging-elevated TNF-α promotes the clonal hematopoiesis of DNMT3A R882H-carrying HSCs (Liao et al., 2022), indicating that a single mutation in an essential gene may yield different output upon TNF-α stimulation.

Given that age-associated increase of inflammation is one of the main driving forces promoting HSCs aging, how does the balance of the immune system stir with aging? It is notable that intestinal epithelial barrier has been remodeled with increased intestinal permeability in old age, and increased TNF-α levels are imperative for this increased permeability. Microbial compounds enter the circulation mediating hematopoiesis by impairing macrophage function and elevating inflammatory factors such as TNF-α (DeGregori, 2020). Otherwise, recent studies have provided new evidence that retrotransposons affect and even promote aging process and age-related diseases. Long interspersed element-1 (L1) is the only autonomous retrotransposable element present in human genome. Increased L1 activates cGAS-STING signaling followed by the increase of the IFN-I response, downstream interferon-stimulated genes (CXCL1 and CXCL10) and other cytokines (IL-6, IL-17A, and TNF-α) in telomere dysfunctional mice and senescent cells, while the repression of L1 alleviates inflammation and furthermore improves the function of HSCs (Wang et al., 2020f). Beyond that, inflammatory storm induced by other aged organs may also lead to HSCs senescence. It has been reported that the age-associated musculoskeletal conditions affect surrounding cells and tissues through the SASP factors such as IL-1, IL-6 and TNFs (Wan et al., 2021).

In addition to the above observations, is there any other signals that change the balance of immune system during aging? A variety of nucleotide species, fragments of telomeric DNA, ruptured micronuclei, CCF, and compromised mitochondria, have been demonstrated to trigger cGAS-STING signaling and construct an inflammatory microenvironment (Gorbunova et al., 2021). Moreover, NOD-like receptor 12 (NLRP12), a member of inflammasome family, maintains the function of HSCs in response to persistent DNA damage and aging (Lin et al., 2020). Following these lines of argumentation, it is intriguing to untangle the interaction among DNA damage, innate immune response and

HSC aging.

Summary

Here, we have summarized phenotypic and functional alterations in aged HSCs, and represent the important factors that lead to HSCs aging, including replication stress and DNA damage, accumulation of toxic byproducts such as ROS, epigenetic modification, HSC microenvironment and inflammation. These biological processes form a complex interaction network to regulate HSCs senescence, although there may be uncharted territory to be explored. We have achieved significant success in the animal model study, but the differences between the animal model and human hematopoietic systems force us to carry out extensive exploration in human system. With the coming of aging society, it is urgent to improve the health care and ensure the life quality with "healthy aging" of the elderly. Deciphering the molecular mechanism of HSC aging will bridge the gap to application in clinical therapeutic targets for reducing the risk of anemia, leukemia, and aging-related diseases.

Human aging, age-related disease, and longevity

Aging in human is an incredibly complex process, characterized by a progressive decline in physiological functions and adaptive capacity, as well as an increase in susceptibility to disease and death. Consequently, how to delay aging and compress age-related disease has become a huge challenge in human society. Here we mainly summarize the current situation of population aging and elderly health and highlight the great potential of longevity cohort as a model in mining the knowledge on human healthy aging.

Aging human population

Over the past century, driven by considerable improvements in hygiene, health care, diet, and nutrition, the global average life expectancy (LE) at birth has greatly increased, climbing from 46.5 years in 1950–1955 to 72.8 years in 2021, according to the World Health Organization (WHO). This extension in lifespan highlights a tremendous advance in public health. However, coupled with a decrease in the fertility rate, it has also resulted in an acceleration of population aging. In 2018, the UN reported that, for the first time in human history, people aged 65 or older outnumbered children under the age of 5 globally. As the most populous country in the world, China is also experiencing a serious aging problem. The World Bank (2018) estimates that by 2050, 26% of China's population will be aged 65 or older. Importantly, and not optimistically, healthy life expectancy (HLE), representing the expected years an individual will live without disease or disability, has not seen the same extension as LE (Crimmins, 2015; Hung et al., 2011). Notably, data suggest that most of older adults live with at least one chronic health condition (Caughey et al., 2008). As a consequence, there is a growing number of people who will enter old age and experience long-term ill health, leading to a massive burden on global health care and society. Nonetheless, further increasing LE, especially HLE, remains one of humanity's great quests and could produce enormous economic and social value (Scott et al., 2021).

Another concern in human aging and longevity is the disparities between women and men. Women generally have a longer LE than men. At present, the global LE for women is 75.6 years and for men is 70.8 years, while in China, it is 79.7 and 75.4 years, respectively. The reasons for these sex-based differences have been widely explored. The compensatory effects of the second X chromosome and protective effects of estrogen have been proposed to explain, at least in part, the female survival advantage (Davis et al., 2019; Mendelsohn, 2002). Increasing evidence also suggests that sexually dimorphic immune responses (e.g., slower rate of decline in immunosenescence in women) are associated with differences in LE (Caruso et al., 2013). Several studies based on age-associated methylation changes have revealed that the lower rates of aging in women are correlated with decreased incidence of CVD compared to men (Horvath et al., 2016; Xiao et al., 2018a). Moreover, women are reported to have a higher abundance of multiple gut microbial species with beneficial effects on host metabolism (Zhang et al., 2021h). However, despite these studies, how to close the LE gap remains elusive.

Aging as a primary driver of age-related chronic disease

Aging itself is the greatest risk factor associated with chronic diseases in human, including CVD, cancer, diabetes, and AD, which have replaced infectious diseases as the leading cause of human death and disability. According to the WHO, ~17.9 million people died from CVD worldwide in 2019, representing 32% of all global deaths. Indeed, researchers have discovered many common features of aging and age-related chronic diseases. For example, the accumulation of genomic mutations in somatic cells throughout a person's life is widely recognized as an important driver of tumorigenesis (Martincorena and Campbell, 2015). Likewise, older individuals exhibit progressive elevation in proinflammatory cytokines, termed "inflammaging", resulting in an increased risk of developing CVD, AD, and other diseases (Ferrucci and Fabbri, 2018; Franceschi and Campisi, 2014). Many other hallmarks of aging, such as mitochondrial dysfunction and cellular senescence, also play crucial roles in various age-related pathologies (Franceschi et al., 2018; López-Otín et al., 2013). Therefore, slowing or inhibiting aging itself is an alternative and more fundamental approach to delay or even treat a variety of age-related diseases in humans.

Human aging and health are highly heterogeneous and are

affected by multiple factors, including genetic, epigenetic, environmental, and stochastic factors (Ahadi et al., 2020; Brooks-Wilson, 2013). Many studies have shown that some genetic variations in the human genome contribute to the susceptibility to age-related chronic diseases (Carrasquillo et al., 2009; Vergeer et al., 2010). Differences in an individual's lifestyle and exposure can lead to epigenetic modifications, which play an important role in aging trajectories and outcomes (Tan et al., 2016). A previous study has suggested that individuals can be classified into different aging patterns, termed "ageotypes", based on biomarkers retrieved from multiomics data (Ahadi et al., 2020). In addition, different tissues and organs in a human body can display heterogeneous aging (Schaum et al., 2020), in part because cells in certain tissues age early, thereby locally or distantly promoting asynchronous aging among tissues (Rando and Wyss-Coray, 2021). Therefore, advanced estimation of age-dependent functional decline in tissues could provide some guidance on delaying age-related diseases.

The identification of molecular biomarkers with consistent age-associated changes across a human lifespan has driven the design of "molecular clocks" to predict an individual's chronological age (CA) (Fleischer et al., 2018; Hannum et al., 2013; Horvath, 2013). Notably, two independently developed DNA methylation (DNAm)-based "epigenetic clocks" show high accuracy in CA prediction (Hannum et al., 2013; Horvath, 2013). More intriguingly, growing evidence indicates that "molecular clocks" are associated with higher biological age (BA) relative to CA (Fransquet et al., 2019; Lehallier et al., 2019; Sayed et al., 2021). BA is a concept that attempts to capture physiological changes along the aging courses, thus seems to be a more meaningful measure to estimate health of a person (Li et al., 2020a). Indeed, the epigenetic age acceleration (i.e., difference between epigenetic age and CA) has been linked to the risk of multiple age-related outcomes (Fransquet et al., 2019; Xiao et al., 2019) and multiple AD risk factors (e.g., high blood pressure) (McCartney et al., 2018). Additionally, a recent study also applied blood immunome data to develop an iAge that can track multimorbidity and estimate BA of a person (Sayed et al., 2021). Thus, these studies confirm the successful utilization of "molecular clocks" in tracking human aging and age-related diseases.

Centenarians-excellent paradigms of healthy aging and longevity in human

It is generally accepted that the maximum lifespan for human is around 125 years (Dong et al., 2016). The current longest human lifespan on record is 122 years, the age at death of Jeanne Louise Calment. Within human society, there is a growing number of people, although total numbers remain small, who have reached the age of 100 (i.e., centenarians), which is considered to be at the extreme limit of human lifespan. Importantly, centenarians display markedly decreased susceptibility to many major age-related diseases (e.g., CVD, AD, and cancer) (Engberg et al., 2009; Hitt et al., 1999). For instance, based on autopsy studies, centenarians are characterized by a significant decrease in cancer prevalence and metastatic rate when compared with the elderly (Pavlidis et al., 2012). Evidence from epidemiological surveys also shows that centenarians exhibit relatively good health upon reaching 100 years of age when compared to their shorter-lived contemporaries at the same time point of living (Ailshire et al., 2015; Engberg et al., 2009). In addition, the siblings and offspring of centenarians also exhibit certain survival advantages, including increased likelihood of longevity and reduced risk of CVD (Perls et al., 2002; Terry et al., 2003), echoing the scenario of moderate heritability (15%–40%) of human longevity and healthy aging (Morris et al., 2019). Thus, centenarians offer the opportunity to identify the characteristics of extreme aging and the potential health-protective mechanisms underlying very old age in humans.

Accordingly, many studies have focused on the identification of longevity-associated genetic variants in long-lived populations, including centenarians (Garagnani et al., 2021; Liu et al., 2021f). As seen in Table 3, several longevity-associated genes and variants have been identified in different longevity cohorts. However, only a few genes, e.g., *FOXO3* and *APOE*, have been consistently associated with human longevity across multiple populations (Christensen et al., 2006). Several studies also suggest that most genetic variants have a very limited effect on human longevity (Passarino et al., 2016). As such, studies have since explored the health-protective mechanisms in centenarians at other molecular levels, including gene expression, DNAm, and gut microbes (Jiang et al., 2021; Sato et al., 2021; Szymczak et al., 2020; Xiao et al., 2018b). For example, by analyzing transcriptome data from peripheral white blood cells of Chinese centenarian families, a recent study has shown that centenarians and their offspring exhibit relatively high autophagy-lysosomal function (Xiao et al., 2018b). Moreover, higher mtDNA content, which may ensure sufficient energy supply, serves as another marker for centenarians (He et al., 2014; He et al., 2016). In addition, it has been shown that centenarian-specific gut microbiota (e.g., Odoribacteraceae strain) can generate unique secondary bile acids (e.g., iso-alloLCA) and have health-protective functions (Sato et al., 2021).

However, despite considerable achievements in centenarian studies, issues involving appropriate control groups remain a challenge in this research field. At present, younger cohorts are still the most widely used control samples in centenarian studies. However, how many will also achieve longevity is difficult to estimate. More importantly, the age gap between centenarians and younger controls greatly in-

Table 3 Genetic variants associated with human longevity[a)]

Variant	Gene name	Longevity allele	Variant position	References
rs2802292	*FOXO3*	G	Intronic	(Anselmi et al., 2009; Willcox et al., 2008)
rs429358-rs7412	*APOE*	ε2	Missense	(Schächter et al., 1994; Sebastiani et al., 2019)
rs34516635	*IGF1R*	A	Missense	(Suh et al., 2008)
rs5882	*CETP*	G	Missense	(Barzilai et al., 2003)
rs7896005	*SIRT1*	A	Intronic	(Kim et al., 2012)
rs1800795	*IL6*	G	Promoter	(Hurme et al., 2005)
rs2075650	*TOMM40*	G	Intronic	(Liu et al., 2021f)
rs17169634	*BMPER*	G	Intronic	(Liu et al., 2021f)
rs3803304	*AKT1*	C$^{(\wedge)}$	Intronic	(Pawlikowska et al., 2009)
rs2755209	*FOXO1*	T$^{(\wedge)}$	Intronic	(Li et al., 2009)

a) "^" indicates that allele is negatively correlated with human longevity.

creases the difficulty in distinguishing aging- and longevity-related factors in centenarians. Nevertheless, emerging evidence suggests that many factors are associated with both the lifespan and helthspan of human beings (Zeng et al., 2020). In addition, the geroprotective effects of some factors may depend on interactions with the environment, which can vary in centenarian and younger cohorts (Willcox et al., 2006). Accordingly, a longitudinal study of an elderly cohort until the age of 100, although challenging, will no doubt provide important knowledge on healthy human aging and longevity. Moreover, a paradoxical phenomenon is consistently observed in modern societies, whereby women live longer but experience higher rates of physical illness than men in later life. This paradox is also observed in centenarian cohorts, with a reported male/female ratio of 1:2–1:7 (Passarino et al., 2002), but with male centenarians experiencing significantly lower morbidity and fewer geriatric syndromes than females (Hazra et al., 2015; Perls, 2017). Thus, male centenarians may be an optimal natural model for deciphering the protective mechanisms that may help men live longer, healthier lives.

Summary

In modern society, human beings have made great achievements in prolonging their LE. Nevertheless, it is a pity that elderly always suffer from one or more age-related diseases for a long time. Consequently, these conditions lead to the rapid aging of the population, which has become an inevitable and urgent global problem and poses unprecedented challenges for public health and economic development. Thus, our focus should be on increasing the HLE of the human population. Prolonging HLE should not only improve the health and quality of life of the elderly population, but also curb healthcare costs and increase labor force. Of note, we here review the findings of the longevity cohort study and emphasize the importance of further longitudinal research on the elderly cohorts until the age of 100, which could express a large number of important clues related to the realization of human healthy aging and might provide effective scientific guidance for solving the problem of aging.

The interventions of aging

Approaches to prevent or intervene with various age-related disease processes and ultimately to prolong healthspan or lifespan are emerging. In this chapter, we provide several strategies with potential for clinical application in geroprotection. Gene therapies targeting cells to delay or reverse some aspects of tissue or systemic aging are introduced, including (1) using gene therapy to change aging-associated genes to extend lifespan or healthspan, (2) transplantation of genetically modified stem cells to rejuvenate aged tissues, and (3) eliminating senescent cells that contribute to the inflammatory milieu in aged organs. We also discuss the application of small molecules, systems biology and artificial intelligence (AI) in aging interventions. Finally, novel technologies to aid in aging research are presented, including new model systems, single-cell omics technologies, imaging-based techniques and computational methods.

Gene therapy as a novel approach to combat aging

A key goal for aging interventions is to extend the healthspan and lifespan. Many geroprotective genes have been identified using transgenic or loss-of-function animal models (Kurosu et al., 2005). With the development of gene-editing technologies over the past two decades, it is feasible to add, delete, or change nucleotide sequences at specific locations in the genome. Recently, gene therapies that can extend the lifespan or healthspan have emerged. To explore this perspective, we summarize recent works on aging interventions using novel gene therapy approaches (Table 4).

In vivo gene therapy for aging interventions

For *in vivo* gene therapy, the first step is to identify the targets.

Table 4 Gene therapy strategies for aging intervention[a)]

Target	Delivery system	Strategy	Administration route	Starting time of intervention*	Frequency of intervention	Lifespan extension	Physiological improvement	References
FGF21, TGFßR2 and αKlotho	adeno-associated virus (AAV) 8	FGF21	retroorbital injection	29-37 weeks old	one dose	N/A	↓obesity and diabetes phenotypes in mice fed with high-fat diet	(Davidsohn et al., 2019)
		FGF21 alone or in combination with TGFßR2 /αKlotho		18 months old	one dose	N/A	↓aging-related obesity	
		FGF21 in combination with TGFßR2		8 weeks old	one dose	N/A	↓renal medullary atrophy in mice subjected to unilateral ureteral obstruction	
		TGFßR2 alone or in combination with FGF21, and αKlotho		6 months old	one dose	N/A	↑heart function in mice subjected to ascending aortic constriction	
Oct4, Sox2, Klf4 (OSK)	AAV2	OSK	intravitreal injection	12 weeks old	one dose	N/A	↓vision loss in mice with glaucoma	(Lu et al., 2020a)
				11 months old (NIA Aged Rodent Colonies)	one dose	N/A	↓aging-related vision loss	
TERT and FST	AAV9	TERT	intravenous injection	12 months old	one dose	median lifespan extension by 24%	↑insulin sensitivity, osteoporosis, neuromuscular coordination	(de Jesus et al., 2012)
				24 months old	one dose	median lifespan extension by 13%		
	mouse cytomegalovirus	TERT	intranasal administration or intraperitoneal injection	18 months old	one dose per month for six consecutive months	median lifespan extension by 41.4%	↑glucose tolerance, physical performance, and ↓loss of body mass and alopecia	(Jaijyan et al., 2022)
		FST		18 months old	one dose per month for six consecutive months	median lifespan extension by 32.5%		
KAT7	lentivirus	CRISPR-Cas9–based KAT7 depletion	intravenous injection	20 months old	one dose	median lifespan extension by 25%	↑overall appearance, grip strength, behavioral response to anxiety	(Wang et al., 2021d)
				around 8 weeks old (*Zmpste24*$^{-/-}$)	one dose	median lifespan extension by 34%	N/A	
VEGF	AAV9	VEGF	intraperitoneal injection	2 or 8 months old	one dose	N/A	↑carbohydrate oxidation and respiratory quotient	(Grunewald et al., 2021)
				8 months old	one dose	N/A	↑rotarod performance associated with microvascular density	
IκBα	lentivirus	dominant-negative IκBα	mediobasal hypothalamus injection	around 18 months old	one dose	maximal lifespan extension by about 10%	↓aging-related cognitive decline and muscle weakness	(Zhang et al., 2013a)
	genetically engineered hypothalamic stem cells	htNSCs derived from newborn mice that stably expressed dominant-negative IκBα	mediobasal hypothalamic implantation	18 months old	one dose	maximal lifespan extension by about 10%	N/A	(Zhang et al., 2017b)
				16 months old	one dose	N/A	↓aging-related physiological declines and cognitive impairment	
FOXO3	genetically engineered vascular cells	FOXO3-engineered human ESC-derived vascular cells	intramuscular injection	8-10 weeks old (BALB/c)	one dose	N/A	↑vascular regeneration and repair	(Yan et al., 2019b)
				4-6 weeks old (NOD-SCID)	one dose	N/A	↑resistance to tumor transformation	
	genetically engineered mesenchymal progenitor cells	FOXO3-engineered human mesenchymal progenitor cells	intramyocardial injection	10-12 weeks old (NOD-SCID)	one dose	N/A	↓left ventricular remodeling and cardiac dysfunction in mice with myocardial infarction	(Lei et al., 2021)
uPAR	T cells	uPAR-directed CAR T cells with combined MEK and CDK4/6 inhibitors	intravenous injection	8-12 weeks old	one dose or one dose per week for two consecutive weeks	maximal lifespan extension by about 5%	N/A	(Amor et al., 2020)
		uPAR-directed CAR T cells	intravenous injection	14-18 weeks old	one dose	N/A	↓chemical-induced liver fibrosis	
				20-22 weeks old	one dose	N/A	↓diet-induced liver fibrosis	
FAP	lipid nanoparticles	FAP-directed CAR T cells	intravenous injection	adult	one dose	N/A	↓cardiac fibrosis and dysfunction caused by injury	(Rurik et al., 2022)

a) * The genetic background of the animal model not-mentioned in the table is C57BL/6. N/A, not assessed.

Previous studies have shown that an aggravated burden of senescent cells may constitute a driving force behind aging and aging-related diseases. Therefore, senescent cells have emerged as a potential target for the prevention or treatment of a variety of age-related diseases, as well as for the extension of healthspan or lifespan. Telomere shortening and DNA damage are well-known causes of cellular senescence and are associated with both physiological aging and various aging-related diseases. As such, one promising target for aging intervention is the *Tert* gene, which encodes telomerase reverse transcriptase (TERT), a ribonucleoprotein polymerase essential for the replication of chromosome termini (the telomeres). One-time tail vein injection of AAV9-*Tert* in 1- or 2-year-old mice delays the onset of age-related pathologies and extends their lifespans by 24% or 13%, respectively (Bär et al., 2014; Bernardes de Jesus et al., 2012). Moreover, a recent study reported that intranasal or intraperitoneal administration of the mouse cytomegalovirus (MCMV) expressing TERT or FST (follistatin, another target to increase muscle mass and to improve the neuromuscular function) in 18-month-old mice once a month for six consecutive months leads to a median lifespan extension of 41.4% or 32.5%, respectively (Jaijyan et al., 2022).

Besides shortened telomeres, senescent cells also secrete a group of proinflammatory factors, called SASP, which impair the function of stem cells, alter the organization of the ECM, and spread the senescence phenotype to neighboring cells, leading to systemic chronic inflammation. NF-κB is a central mediator of inflammation and is considered as a primary regulator of SASP. Therefore, inhibiting NF-kB activation holds the potential to combating aging via reducing inflammation. In this respect, IκBα (the NFKB inhibitor alpha) has been identified as a potential therapeutic target. One study in the hypothalamus, a key organ for systemic aging, found that targeted injection of lentivirus expressing dominant-negative IκBα into the hypothalamus inhibits NF-κB activation and thereby increases mouse maximal lifespan by approximately 10% (Zhang et al., 2013a).

In addition to the genes discussed above, a pioneering work recently identified new aging intervention targets using a genome-wide CRISPR-based screen in the human stem cell senescence model (Wang et al., 2021d). A top hit in this screen is lysine acetyltransferase 7 (KAT7), a histone acetyltransferase, whose deficiency alleviates cellular senescence in human mesenchymal precursor cells. Indeed, single-dose tail vein injection of lentiviral vectors encoding Cas9/sg-*Kat7* in mice attenuates liver aging. It also extends the median lifespan of physiologically aged mice and progeroid mice by 25% and 34%, respectively. This study contributes additional gene targets for aging interventions and offers new gene therapy strategies to combat cellular senescence and the decline of organ function throughout aging.

As the largest cellular network shared by all organs, vascular aging is also believed to be a driving force of aging. Therefore, *Vegf*, encoding vascular endothelial growth factor (VEGF), which serves as a key mediator of angiogenesis, is another target gene for aging intervention. Introduction of VEGF may regenerate or repair blood vessels to meet the needs of vascular supply in tissues. Indeed, adeno-associated virus (AAV)-mediated *Vegf* transduction into 8-month-old mice increases carbohydrate utilization and respiratory quotient, accompanied by the amelioration of muscle loss and better preservation of muscle-generating force (Grunewald et al., 2021).

Way beyond the action of individual genes, aging is a complicated, multi-stage process characterized by progressive degeneration and loss of function in various organs and tissues. This process is attributed to complex interactions between many intrinsic and extrinsic factors. In this regard, gene therapies that target multiple geroprotective factors simultaneously (i.e., combinatorial gene therapy) may lend more strength to geroprotection. For example, fibroblast growth factor 21 (FGF21) is a secreted peptide hormone that binds to the FGF receptor (FGFR) and acts on multiple tissues to regulate energy homeostasis, rendering it an intervention target for metabolic syndromes (Jimenez et al., 2018). Additionally, the binding of FGF21 to FGFR requires the co-receptor Klotho, which is a regulator of intracellular calcium and a geroprotective factor for the kidney and heart (Davidsohn et al., 2019). Transforming growth factor β1 (TGFβ1) is a ubiquitously secreted factor, and its signal transduction is implicated in hypertrophic cardiomyopathy (HCM), immune regulation, and ECM organization. Synergistic advantages for aging intervention are demonstrated by co-expression of FGF21, TGFβ receptor 2 (TGFβR2), and αKlotho (KL) (Davidsohn et al., 2019). Taking advantage of AAV vectors to mediate long-term production of these therapeutic proteins, researchers have developed a combinatorial gene therapy co-expressing FGF21, TGFβR2, and KL to alleviate multiple aging-associated organ defects, including HCM, abnormal immune recruitment, and disorganized ECM formation in adult mice fed with a high-fat diet (Davidsohn et al., 2019). Another example is the "well-known" Yamanaka factors (*Oct4*, *Sox2*, *Klf4*, and *c-Myc*), which have recently been used to rejuvenate aged cells or tissues. This combination can reconstitute the epigenetic state of terminally-differentiated cells, transform mature somatic cells into stem or progenitor cells, and rejuvenate aged cells to a younger state. Indeed, studies show that cyclic induction of Yamanaka factors, either in the short- or long-term, can alleviate cellular phenotypes associated with aging and prolong the lifespan of progeroid mice (Browder et al., 2022; Chondronasiou et al., 2022; Ocampo et al., 2016). Notably, ectopic expression of three specific genes (e.g., *Oct4*, *Sox2*, and *Klf4*) is sufficient to reverse vision loss in aged mice (Lu et al., 2020a).

Transplantation of genetically engineered cells for aging interventions

Aging is associated with a decline in organ and tissue repair and regeneration abilities. This deterioration is due to a decreased reservoir or function of stem cells. Therefore, in addition to targeting specific genes, another way to antagonize aging is to replace "bad" cells within old organisms directly by transplanting "good" cells. This stem cell transplantation therapy holds therapeutic promise for restoring homeostasis in aged organs. However, aged organs are usually characterized by a detrimental microenvironment that is not favorable to maintain stem cell homeostasis. To deal with this problem, genetically enhanced stem cells are required for transplantation, endowing stem cells with greater regenerative ability and stress resistance through genetic enhancement strategies to fight against the deleterious aging microenvironment. For example, hypothalamic stem cells experience substantial loss during aging, and transplantation of genetically engineered hypothalamic stem cells (expressing dominant-negative IκBα), which are capable of surviving in the aging microenvironment, extended the lifespan by 10% in mice when carried out at ~18 months of age (Zhang et al., 2017b). In addition to adapting to the unfavorable microenvironment, safety and effectiveness are both of prominent importance for the application of these genetically engineered cells in translational medicine. In this regard, Forkhead box O3 (FOXO3), a transcription factor associated with longevity, has been selected for stem cell engineering. FOXO3 is specifically silenced in aged arteries (Zhang et al., 2020d), while FOXO3-engineered human ESC-derived vascular cells exhibit delayed senescence, increased resistance to oxidative stress, and most importantly, augmented vascular regeneration and repair ability, alongside an enhanced resistance to tumor transformation (Yan et al., 2019b). Likewise, intramyocardial transplantation of FOXO3-engineered human mesenchymal progenitor cells can also alleviate left ventricular remodeling and cardiac dysfunction in a mouse model of myocardial infarction (Lei et al., 2021).

In addition to refueling or rejuvenating damaged stem cells, another geroprotective strategy is to eliminate abnormally accumulated senescent cells, which produce an inflammatory milieu leading to organ defects. Urokinase-type plasminogen activator receptor (uPAR)-directed CAR T cells are specifically engineered to target and deplete senescent cells (Amor et al., 2020). Intraperitoneal injection of uPAR-directed CAR T cells can extend the survival time of mice carrying lung adenocarcinoma and reduce chemical- or diet-induced liver fibrosis. Notably, efficient delivery of specific mRNA encoding the CAR to T cells is sufficient to generate potent CAR T cells *in vivo* (Rurik et al., 2022). These *in situ* CAR T cells generated using gene therapy reduce cardiac fibrosis and dysfunction caused by injury (Rurik et al., 2022). This collection of emerging technologies paves the way for the development of aging interventions in the near future.

Summary and perspectives

With the advancement of gene editing technologies, the need to develop novel gene therapies for aging intervention has become a compelling focus of the aging research community. Different approaches, including local or systematic administration of gene therapy vectors or genetically engineered cells, will alleviate aging phenotypes or extend the lifespan. However, a more systematic and in-depth understanding of aging and its regulatory mechanisms is required to develop safer and more effective therapies. Besides, targeted delivery strategies for gene therapies, including viral-based systems or non-viral vectors via lipid nanoparticles remain a significant challenge (Wei et al., 2022b). To a certain extent, this has generated limitations to gene therapy applications. Taken together, although exciting, there is still a long way to go before these approaches can be translated into clinical practice.

Aging and drug discovery

The beautiful desire for longevity has never changed for hundreds to thousands of years. With the improvement of society and medical conditions, people live longer, but the aging-associated problems worsen. Aging is associated with many diseases such as AD, amyotrophic lateral sclerosis, cardiovascular disease, and diabetes, which tremendously compromise the life quality of the elderly. Thus, except for living longer, people also desire to live healthier. Over the last decade, scientists have made great efforts to develop drugs to fight against aging, aiming to mitigate multiple age-related diseases altogether.

With the continuous deepening of aging research, many small molecules have been reported to delay aging and at the same time deepen the understanding of the aging process and mechanism. However, aging is a complex process resulting from multiple factors. The exact mechanism remains unclear. Currently, widely accepted intervention targets include NAD^+ supplementation, VEGF supplementation, AMPK, elimination of senescent cells, improvement of inflammation, FOXO family, insulin/IGF-1 signaling pathway, PI3K-AKT signaling pathway, p16, p21 and so on (Childs et al., 2017; Grunewald et al., 2021; Li et al., 2022; López-Otín et al., 2013). The existing geroprotective drugs are screened based on these targets. We list here the current major small molecules and their primary targets for aging intervention.

Senolytics

The accumulated senescent cells are observed in aged tissues and lead to many chronic diseases, such as diabetes, neuro-

degeneration diseases, and so on (van Deursen, 2014). Previous studies have indicated that elimination of senescence hallmarks can lead to extending healthspan in animal models (Baker et al., 2011). These discoveries bring us a new strategy against aging, that is to use senolytic molecules to eliminate senescent cells. In the past decade, several senolytics are reported to specifically eliminate senescent cells and extend lifespan and healthspan. Among them, ABT-263 is the first reported specific and broad-spectrum senolytic drug, which was originally developed as a BCL2 and BCL-XL inhibitor to induce apoptosis in cancer cells. Oral administration of ABT-263 depletes senescent cells and results in the rejuvenation of aged tissue stem cells (Chang et al., 2016). An HSP90 inhibitor 17-DMAG can also target senescent cells to extend healthspan and delay age-related symptoms (Fuhrmann-Stroissnigg et al., 2017). Cardiac glycoside including digoxin and ouabain, which targeted the Na^+/K^+ ATPase, caused the depolarization and acidification of senescent cells that accumulate after irradiation or in old mice (Guerrero et al., 2019; Triana-Martínez et al., 2019). p53 loss-of-function causes chromosomal instability, leading to senescence or apoptosis (Mijit et al., 2020). Therefore, stabilizing p53 points to another way to develop new senolytics. In the past decade, several such strategies have been developed, including FOXO4-D-retro-Inverso (FOXO4-DRI) peptides, which can disrupt the interaction between FOXO4 and p53 (Baar et al., 2017); UBX0101 and RG7112, which inhibit the interaction between MDM2 (the negative regulator of p53) and p53 to restore p53 activity (Chauhan et al., 2012; Jeon et al., 2017). Additionally, a designed prodrug SSK1 targeting SA-β-gal can induce senescent cells into apoptosis and reduce senescent cells in different tissues of aged mice (Cai et al., 2020). To this end, several senolytics have entered into clinical trials and show geroprotective effects. However, senolytic also faced challenges such as cell-type dependent selectivity and possibly increased risk of cancer by interfering with the cell cycle. There is still a long way to develop senolytics as a drug.

NAD-boosting strategies and sirtuin activator

NAD^+ is an important coenzyme for redox reactions, establishing the critical role of NAD^+ in cell metabolism. It is also a substrate for NAD-consuming enzymes, which regulate important cellular functions, including genomic stability, mitochondrial homeostasis, adaptive stress responses, and cell survival (Lautrup et al., 2019). Aging is always accompanied by NAD^+ decline, especially in neurodegeneration diseases. This phenomenon caught our eyes to focus on NAD^+ as a potential treating strategy towards aging. Elevating the NAD^+ level by supplementing NAD^+ precursors such as NR, NMN and NAM is a simple and direct way. Up to now, several clinical trials have been conducted and demonstrated that NMN and NR administration is safe and can efficiently increase NAD^+ levels in healthy volunteers (Covarrubias et al., 2021). Except for supplementation of NAD^+ precursors, modulation of NAD metabolism offers another way to boost NAD^+. To this end, both the enhancement of NAD biosynthesis and the inhibition of NAD consumption have been used. There are three major pathways to generate NAD^+, including *de novo* biosynthesis, the Preiss-Handler pathway, and the salvage pathway. NAMPT is the rate-limiting enzyme in the NAD^+ salvage pathway, which is the primary source of NAD in mammals. The pharmacological evidence from the discovery of the P7C3 molecules demonstrates the benefits of stimulation of NAMPT in protecting damaged or diseased neurons, and probably aged animals (Wang et al., 2014a). NMNATs take part in all the NAD^+ biosynthetic pathways, thus are also considered as a potential target in NAD-boosting strategies (Gu et al., 2022; Liu et al., 2018b). Moreover, TES-991 and TES-102524 target α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD), an enzyme that limits NAD^+ *de novo* pathway, to elevate NAD^+ level in kidney, liver and brain (Katsyuba et al., 2018). On the other hand, reducing NAD^+ consumption is another method to elevate the NAD^+ level. NAD^+-consuming enzymes such as CD38, BST1, PARP, and SARM1 have a vital role in age-related NAD^+ decline. CD38 inhibition has a positive effect on NAD^+ elevating. For example, 4-Amino-quinolines including 78c, 1ah, and 1ai can competitively inhibit CD38 NADase activity (Chini et al., 2018). Treatment with 78c in old mice prevents the age-related NAD decline and alleviates aging symptoms (Tarragó et al., 2018). Some flavonoids molecules including apigenin, Luteolinidin, and Kuromanin also can inhibit CD38 NADase activity (Chini et al., 2018). SARM1 plays an important role in axon degeneration. SARM1 has a TIR domain harboring a NADase activity (Jiang et al., 2020). SARM1 activation is required to execute axon degeneration in response to injury in both *Drosophila* and mice (Gerdts et al., 2013; Osterloh et al., 2012). This made SARM1 a potential target to treat neurodegenerative disorders. Isoquinoline is a selective and potent inhibitor of SARM1, which appears to inhibit the SARM1 NADase activity and allows the protection of the injured axon (Hughes et al., 2021). This molecule may have similar effects on the aged nervous system.

Many geroprotective benefits of NAD replenishment are thought to act through sirtuins. Sirtuin is a family of highly conserved deacetylases, which is comprised of seven members in mammals (SIRT1-SIRT7) (Ji et al., 2022). Sirtuins modulate multiple cellular processes including transcription, metabolism, inflammation, and stress response (Bonkowski and Sinclair, 2016). Because of these important roles, the malfunction of sirtuins has been implicated in many diseases. Sirtuin deacetylase activity depends on NAD^+ as a cofactor. Under aging conditions, NAD^+ decline correlates with the reduction of the sirtuin activities (Braidy et al., 2011). On the

other hand, NAD-boosting can activate sirtuin activities. For example, NMN and NR boost NAD^+ levels and activate SIRT2 activity in *C. elegans* (Mouchiroud et al., 2013). This evidence has led to the hypothesis that direct sirtuin activators may delay the aging process. Resveratrol is the first reported SIRT1 activator, it is also found as a histone deacetylase (HDAC) inhibitor. Activating AMPK and SIR1 signaling pathways are reported to extend lifespan in yeast, nematodes, fruit flies significantly, and fish (Collins et al., 2006; Howitz et al., 2003; Lam et al., 2013; Pearson et al., 2008; Valenzano et al., 2006). However, resveratrol does not extend the lifespan of mammals but only improves their healthy lifespan (Fernández and Fraga, 2011; Miller et al., 2011; Pearson et al., 2008). Therefore, the geroprotective effect of resveratrol needs long-term inspection and evaluation. Epigenetic modification is one of the critical signs of aging. Epigallocatechin gallate (EGCG) can prolong the lifespan of rats. This effect has previously been attributed to its antioxidant and anti-inflammatory properties (Niu et al., 2013). However, in the latest study, the efficacy of EGCG as a HAT inhibitor is reported, proving that acetyltransferase inhibition prolongs lifespan more significantly than the use of antioxidants. At the same time, the results of human cell lines further show that HAT inhibitors are effective against aging in different species (Zhu et al., 2020).

IGF1 signaling inhibitor

IGF1 signaling is a highly conserved pathway that plays a vital role in tissue growth and nutrient sensing. Levels of IGF1 signaling decrease with age. However, it has long been established in different species that reducing IGF signaling can extend lifespan. daf-2 is the homolog of IR-IGF1 in *C. elegans*, and the daf-2 mutant exhibits a significantly extended lifespan (Kenyon et al., 1993). The effect of inhibiting IGF1 signaling on extending lifespan is conserved in yeasts, worms, flies, mice, and humans (Holzenberger et al., 2003; Tatar et al., 2001). This provides us with a promising strategy towards aging, even though the mechanism of IGF1 signaling in extending lifespan remains unclear.

Some molecules for aging intervention have been shown to regulate IGF1 signaling. For example, polyamine spermidine reduces the insulin/IGF signaling, and spermidine supplementation in the diet can extend the lifespan of yeasts, nematodes, fruit flies, and mice and increase healthy lifespan (Eisenberg et al., 2009; Eisenberg et al., 2016; Yue et al., 2017). Acarbose treatment can also decrease IGF1 levels in both males and females and significantly extend the median lifespan in males (Harrison et al., 2019). However, because of the important role of IGF1 signaling in development, direct targeting IGF1 receptors remains a high risk. Thus, deciphering the role of the IGF signaling pathway in the regulation of the human lifespan is needed for drug development.

mTOR inhibitor

As the central controller regulating cell metabolism, growth, proliferation, survival, and autophagy, mTOR is related to many diseases (Chen and Zhou, 2020). mTOR is considered an appealing target to mitigate age-related diseases owing to its central roles in regulating lifespan and aging. It reacts to multiple signals such as nutrients and growth factors to mediate cell growth and metabolism. Reduced mTOR signaling extends lifespan in multiple model animals including yeast (Powers et al., 2006), *C. elegans* (Jia et al., 2004), *Drosophila* (Kapahi et al., 2004), and mouse (Lamming et al., 2012). Rapamycin is a well-known inhibitor of mTOR and has been used as an anti-cancer drug and immunosuppressant. Emerging evidence have demonstrated that treatment with rapamycin is beneficial in extending the lifespan of mice (Bitto et al., 2016; Miller et al., 2011; Neff et al., 2013). Other studies have found that rapamycin can treat or delay many aging-related diseases, such as skin aging, neurodegenerative diseases, muscle aging inhibition, muscular dystrophy prevention, cardiac hypertrophy, diastolic dysfunction, ovarian function decline, immune aging, liver disease, focal bullous fat deposition, myocardial nucleus size, endometrial cystic hyperplasia, and adrenal tumors (Chen et al., 2009; Chung et al., 2019; Dai et al., 2014; Dou et al., 2017a; Flynn et al., 2013; Ham et al., 2020; Kaeberlein and Galvan, 2019; Majumder et al., 2012; Van Skike et al., 2020; Wilkinson et al., 2012). Consistent with the pleiotropic feature of mTOR modulation, the aging process appears to be delayed upon mTOR inhibition in the CNS, immune system, and cardiac system in mice (Halloran et al., 2012; Li et al., 2019b; Reifsnyder et al., 2018).

Currently, rapamycin is not used as a geroprotective drug in the clinical setting because of the side effect including hyperglycemia, hyperlipidemia, and organ toxicity. A pilot trial study has demonstrated that short-term rapamycin treatment (8 weeks) in healthy older human cohort appears to be safe (Kraig et al., 2018). Several mTOR inhibitors, including rapamycin analog, ATP-competitive inhibitors, mTOR/PI3K dual inhibitors, synthetic mTOR inhibitors, and several natural products, have been investigated (Chen and Zhou, 2020). A synthetic mTOR inhibitor Palomid 529 shows little systematic toxicity (Dalal et al., 2013). The effect of these mTOR inhibitors in the context of aging remains to be extensively assessed.

AMPK activator

AMPK maintains cell energy balance by affecting multiple links of cell material metabolism and is considered as an energy sensor. It has been proven in different models that activating AMPK can prolong lifespan (Burkewitz et al., 2014). Metformin is a drug for the treatment of type 2 diabetes. It reduces diabetic hyperglycemia by inducing glycolysis and improving insulin sensitivity (He et al., 2009).

Studies have shown that metformin prolongs the lifespan of *C. elegans* by activating the AMPK pathway. Studies in mice have found that adding 0.1% metformin to the diet can extend the lifespan of mice, while the lifespan of mice with 1% metformin is shorter (Martin-Montalvo et al., 2013). Clinical studies and epidemiological analysis have shown that metformin has beneficial effects on health lifespan (Barzilai et al., 2016; Campbell et al., 2017). At the same time, researchers found that if metformin is provided at the end of their lifespan in nematodes and primary human cells, metformin will shorten their lifespan (Espada et al., 2020). The side effects of metformin are also apparent. Some patients will experience diarrhea, bloating, etc. (Soukas et al., 2019).

Aspirin has been proven to extend the lifespan of nematodes through AMPK (Ayyadevara et al., 2013; Wan et al., 2013). Aspirin can also extend the lifespan of fruit flies and male mice (Song et al., 2017a; Strong et al., 2008) and improve the healthy human lifespan (Cao et al., 2016). However, aspirin also increases the risk of gastrointestinal bleeding (McNeil et al., 2018).

α-KG, as a key metabolite of the tricarboxylic acid cycle, plays a vital role in cell energy metabolism (Harrison and Pierzynowski, 2008). Studies have shown that α-KG can prolong the lifespan of two species of *C. elegans* and *Drosophila* by activating AMPK (Chin et al., 2014; Su et al., 2019). α-KG-fed mice can inhibit chronic inflammation, improve aging-related phenotypes and extend their lifespan (Asadi Shahmirzadi et al., 2020). However, more data and research are still needed to support the impact of α-KG on the lifespan and healthy lifespan of mice and humans.

Berberine (BBR) is a natural alkaloid found in Coptis, and it is commonly used as a dietary supplement for the treatment of diarrhea. Studies have shown that berberine can improve cognitive deficits and muscle dysfunction by activating the AMPK/SIRT1/PGC-1a pathway in the skeletal muscle of naturally aging rats. Berberine prolongs the lifespan of wild-type *Drosophila* pupae, yeasts, and mice (Dang et al., 2020; Navrotskaya et al., 2014; Yu et al., 2018b). It has also been reported that berberine has a variety of pharmacological effects, including blocking fatty liver and obesity caused by a high-fat diet and treating cognitive decline caused by cancer, Parkinson's disease, Alzheimer's disease, diabetes, and diabetes (Cai et al., 2016; Lee et al., 2006; Li et al., 2018b; Sun et al., 2018; Wang et al., 2021f; Zhou et al., 2021). Clinical studies have shown that oral berberine (1 gram per day for three months) can reduce cholesterol, low-density lipoprotein, and triglycerides in patients with hyperlipidemia by 20% to 35% (Kong et al., 2004). This result was further confirmed by animal experiments on a hyperlipidemia golden hamster model (Kong et al., 2004). However, more research is needed to explore the specific mechanism of action of this drug and the possibility of extending lifespan in human.

Inositol

Like NAD^+, inositol level also decreases with aging. A recent screen for lifespan extending endogenous metabolites has found that supplementing inositol to worms increases their lifespan and healthspan through activating daf-18/PTEN and enhancing mitophagy. Similar healthspan effect can also be observed in mice (Shi et al., 2020b).

The above studies have brought hope for delaying aging, but none of these small molecules alone extend lifespan in mice by more than 50% and that the effects are often gender-specific. Furthermore, most of these small molecules are natural compounds and are considered as supplements, not medication.

Stem cell geroprotectors

In addition to geroprotective drugs such as rapamycin and metformin, recently, a group of small molecules are rediscovered to have functions in rejuvenating senescent human stem cells, hence named stem cell geroprotectors (Chen et al., 2021d; Geng et al., 2019b; Kubben et al., 2016; Liu et al., 2022a; Liu et al., 2022e; Shan et al., 2022). For examples, Oltipraz, an antischistosomal agent, has a geroprotevitve effect by activating NRF2-mediated antioxidative response pathway in MSCs differentiated from iPSCs generated from HGPS patient fibroblasts (Kubben et al., 2016). Treatment with Vitamin C, a well-known reducing agent, alleviates premature senescence of WRN-deficient stem cells (Li et al., 2016b). WM-3835, a lysine acetyltransferase KAT7 inhibitor, functions in downregulating the expression of SASP genes and alleviating human MSC senescence (Wang et al., 2021d). Recently, using large-scale chemical screening, researchers have identified small compounds such as quercetin and gallic acid as geroprotective agents against senescence in MSCs (Geng et al., 2019b; Shan et al., 2022). Long-term low-dose quercetin administration has been shown to benefit healthspan in mice (Geng et al., 2019a). Furthermore, supplementation with uridine, an endogenous metabolite that is highly expressed in tissues with strong regenerative capacity or in early life, has been shown to rejuvenate senescent human stem cells, promote multiple tissues regeneration in mice and improve athletic abilities in aged mice (Liu et al., 2022e). The identification of these stem cell geroprotectors opens new avenues for aging intervention.

Summary

Significant progress has been made toward understanding the aging process and developing aging interventions. We summarize the potential drugs for aging intervention described in Table 5. It is exhilarating to witness the improvement of senolytics, but more animal experiments and clinical trials are needed to validate their safeness and geroprotective potentials. NAD replenishment is a plausible strategy in slowing the aging processing and mitigating a

wide spectrum of diseases. Moreover, stem cell geroprotectors are gradually revealing their age-defying potentials. Much work remains to be done to understand the mechanisms by which these molecules intervene in aging, and more clinical trials are needed to determine the indications, dose, and timing of administration. Despite tremendous challenges (e.g., the long-term safety, undefined aging biomarkers, and generally complicated clinical trials), academic organizations, industry, clinical settings, and funding bodies are endeavoring to develop next-generation drugs to fight against aging and improve health of the increasing elderly population.

Systems biology and AI in aging intervention

Aging refers to the decline of the body's fitness and physiological functions and affects a person's lifespan. Although aging is inevitable, its speed and trajectory are variable. According to the "State of World Population 2021" annual report, the elderly population aged 65 and above accounts for 9.6% of the total population that year, while the birth rate falls, and the world′s aging process will further increase. Aging is also the major risk factor for cancer, CVD, neurodegenerative diseases, and other chronic diseases, but research on aging and aging-related diseases is still immature. Research shows that compared with eradicating single diseases, research on aging may bring more significant benefits, and delaying aging can make health and longevity coexist (Scott et al., 2021). A series of animal model studies have also shown that improving aging-related diseases and prolonging lifespan is feasible. The existing strategies to delay aging include regenerative medicine, drugs, blood transfusion therapy, gene therapy, and changes in diet and lifestyle, such as ketogenic diet, calorie restriction, an appropriate increase in exercise, etc. Still, these strategies individually are not enough to prevent senile diseases (Partridge et al., 2018). At the same time, there are many difficulties in controlling diet and lifestyle, but regenerative medicine, blood transfusion therapy, and gene therapy are difficult to achieve in human. Therefore, the development of aging intervention drugs has become an important strategy to delay aging.

AI and aging intervention drugs

At present, there are still considerable challenges in aging intervention and drug research. (1) The drug development time and economic cost are enormous, especially for geroprotective drugs. Research on aging takes longer, and model organisms cannot fully simulate the physiological and pathological changes in the human body's aging process.

Table 5 Major geroprotective drugs described in the text

Drug Category	Drug targets	Example drugs	References
Senolytic	Bcl-2 and Bcl-xl	ABT-263	(Chang et al., 2016)
	HSP90	17-DMAG	(Fuhrmann-Stroissnigg et al., 2017)
	Na^+/K^+ ATPase	Digoxin	(Guerrero et al., 2019)
	FOXO4-p53	FOXO4-DRI	(Baar et al., 2017)
	MDM2-p53	RG7112	(Chen et al., 2021c)
	SA-β-gal	SSK-1	(Cai et al., 2020)
NAD^+ precursors	–	NR	(Covarrubias et al., 2021)
		NMN	(Covarrubias et al., 2021)
		NAM	(Covarrubias et al., 2021)
NAD metabolism modulator	NAMPT[a)]	P7C3	(Wang et al., 2014a)
	ACMSD[b)]	TES-991	(Katsyuba et al., 2018)
NAD^+-consuming enzyme inhibitor	CD38	78c	(Chini et al., 2018)
	SARM1	Isoquinoline	(Hughes et al., 2021)
Sirtuin activator	SIRT1	Resveratrol	(Baur et al., 2006)
IGF1 signaling inhibitor	IGF1 signaling	Polyamine spermidine	(Tain et al., 2020)
		Acarbose	(Harrison et al., 2019)
mTOR inhibitor	mTOR	Rapamycin	(Kraig et al., 2018)
		Palomid 529	(Dalal et al., 2013)
AMPK activator	AMPK pathway	Metformin	(Campbell et al., 2017)
		Aspirin	(Cao et al., 2016)
HAT inhibitor	HAT[c)]	EGCG	(Daniel and Tollefsbol, 2015)
daf-18/PTEN activator	daf-18/PTEN	Inositol	(Shi et al., 2020b)

a) NAMPT, Nicotinamide Phosphoribosyltransferase. b) ACMSD, α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase. c) HAT, Histone acetyltransferases.

Therefore, the research results of model organisms may not be successfully translated into human. (2) Although various omics-data trained aging clocks have been developed (Xia et al., 2021), most of clocks or the existing biomarkers cannot represent the health status of the entire organism. Evaluating changes in aging requires a comprehensive set of robust biomarkers to combat aging more accurately (Zhavoronkov and Bhullar, 2015). (3) The aging mechanism is complicated. It is not clear how many "aging phenotypes" or "ageotypes" (Ahadi et al., 2020) there are. The human body is a complex system composed of billions of independent cells that form different tissues and organs, and dysfunctions that affect only a few biological processes in one or more organ can spread to all body parts. The complex interactions between environmental, mechanical, biochemical, and evolutionary constraints also affect aging. With the continuous emergence and advancement of new experimental technologies, a large amount of data related to aging have also been accumulated through the next-generation sequencing- and imaging-based approaches, enabling the characterization of genome-wide epigenomics, transcriptomics, proteomics, and even metagenomics landscapes to further delineate the physiological and pathological aging (Alexander et al., 2017; Bolotin et al., 2017; Nielsen, 2017; Zabolotneva et al., 2013; Zhu et al., 2021a). These data now enable us to use systems biology approaches to study aging, and using AI to screen geroprotective drugs.

Systems biology and AI in aging research

From the systems biology perspective, aging can be viewed as a trajectory diverging from the young resilient state and moving to an old state that is less resilient to stress and challenge. Despite the universality of aging, the rates and trajectories of aging for each individual are not necessarily the same. For example, aging-related diseases move the system into an aging trajectory that is alternative to the healthy aging trajectory, or longevity trajectory. Whether these trajectories can be predicted ahead of time and whether enhanced aging rate is predictive of a divergent trajectory is also not known.

Aging clocks refer to linear or non-linear models based on omics data, trained on chronological or biological ages. Many aging clocks have been developed in recent years to measure aging rate of aging. These include methylation clocks (Hannum et al., 2013; Horvath, 2013), transcriptome clocks (Peters et al., 2015), immune clocks (Sayed et al., 2021), facial clocks (Chen et al., 2015c; Xia et al., 2020), psychological clocks survey (Zhavoronkov et al., 2020) and gut microbiome (Galkin et al., 2020), and so on (Jansen et al., 2021; Johnson et al., 2021; Ryan, 2021; Xia et al., 2021). Similar methylation-based clocks have also been developed for mice (Petkovich et al., 2017). With the advent of AI technology and the ever-growing size of the omics data, more and more of these clocks are now based on AI rather than linear statistical models. For example, an inflammatory cytokine panel trained AI model has been used to define multiple diseases and organ conditions (Sayed et al., 2021); The 3D facial image based AI models have been used to identify lifespan impacts on aging rates and the potential mediators in the blood transcriptome (Xia et al., 2020); large psychological surveys (Zhavoronkov et al., 2020) and gut microbiome (Galkin et al., 2020) based AI models has also been used to access the overall healthy aging. With the development of AI, imaging, omics, epigenetics, blood markers, etc., will be combined to comprehensively predict a panoramic view of an individual's biological age.

These clocks are often not synchronized, some tissues appear to age faster than other tissues (Ghanam et al., 2019; Horvath, 2013). However, there was significant consistency between the two aging rates among the fast and slow aging outliers (Xia et al., 2020). The aging rate analyses based on various clocks already suggest that different tissues are not aging at the same rate, and might not start at the same time, but most of such studies cross section analyses require large scale longitudinal analyses to confirm, and to verify the rate and temporal order of tissue aging. A small longitudinal study using multiomics signatures defines that 4 ageotypes are not always synchronized within the same individual (Ahadi et al., 2020). These asynchronization but eventual consistency of the clocks at different levels or in different tissues indicate the complex interactions among different tissues to drive aging. Indeed, a recent study shows that immune system aging alone drives solid organs and aging of the whole system. Consistently, parabiosis studies show that the young or old blood rejuvenates or ages many tissues and organs of the reciprocal recipients (Ma et al., 2022).

Aging clocks also reveal large heterogeneity of aging across different individuals. For example, the human facial aging clock and the blood transcriptome clock both show a peak of heterogeneity in aging rate between 40 and 50 years old, suggesting middle age is an important window for personalized aging intervention. Different lifestyles of the individuals can contribute the aging rate heterogeneity at least through inflammatory status in the blood (Xia et al., 2020). Other dimensionality and longitudinal data are still needed to corroborate this finding.

Many studies on aging transcriptomes have shown that, on average, aging trajectories are a continuation of developmental trajectories and can be delayed by lifespan-extending interventions. However, different trajectories may exist across different individuals. For instance, during the replicative aging of each single yeast cell, two distinct trajectories exist, and one results in a significantly longer lifespan than the other. More interestingly, genetically modifying the regulators of trajectories results in a new aging trajectory with much longer lifespan than either of the natural trajec-

tories (Li et al., 2020b). Such studies highlight the potentials using a combinatory or systems approach to target the regulatory network to maximize healthy aging and longevity.

Indeed, regulatory network generated or inferred from large experimental data are now enabling computational modeling of the complex processes, such as aging (Han, 2008). When combining network structure inference from transcriptome profiles and network dynamics simulation, researchers uncover the key aging regulatory circuitry that is synergistically targeted by dietary restriction and intermittent fasting in *C. elegans* to delay aging; mildly but synergistically boosting multiple nodes in this network, AMPK, TOR and IIS pathways allows the network to stay more frequently in the young rather than the old steady state; the triple perturbation is so efficient that the lifespan under this condition is 3 times of the wild type (Hou et al., 2016). Furthermore, as shown by Juan Carlos Izpisua Belmonte, Tom Rando, David Sinclair and others' groups, the remarkable rejuvenation effects of the combination of four Yamanaka factors ultimately pave a way to reverse aging at the systems level.

Employ AI to screen drugs based on the generalization and individualization of aging

The existing drug screening is based on a specific target, and aging does not have a single target protein. Many known targets have been curated in the following databases. CellAge (http://genomics.senescence.info/cells/) is a genetic database related to cell senescence, including various resources, such as LongevityMap (http://genomics.senescence.info/longevity/). LongevityMap is a human longevity genetic association knowledge base (Tacutu et al., 2018). GenAge (http://genomics.senescence.info/genes/) is a benchmark database of age-related genes. The Geroprotectors (http://geroprotectors.org/) database contains more than 250 life-extension experiments on 11 wild-type model organisms and data on more than 200 longevity-promoting chemicals, including compounds that have been approved for human use (Moskalev et al., 2015). Aging Atlas (https://ngdc.cncb.ac.cn/aging/index) is a multiomics database including aging-related genes, transcriptomics, epigenomics, single-cell transcriptomics, proteomics and pharmacogenomics and metanolomics for aging biology (Liu et al., 2021). Currently an increasing number of small molecule transcription profiles have been generated to promote the development of many drug molecules (Segler et al., 2018; Subramanian et al., 2017). A recent research uses the simplified molecular-input line-entry system (SMILES) chemical code as input to construct a neural network that fits the small molecule transcription profile measured in the L1000 project, and validates the predictions in many diseases (Zhu et al., 2021a). Another advantage of AI is that it can design new molecules based on multi-pharmacological principles and help to generate safer drug molecules (Reddy and Zhang, 2013). At present, AI has begun to be applied to the screening of geroprotective drugs (Kapsiani and Howlin, 2021), but it is only in its infancy and still has broad prospects. Meanwhile, the characteristics used in analyzing the aging clock can be used to screen compounds to delay aging. These clocks also provide convenience for drug efficacy evaluation and shorten the development cycle. With the advent of the digital age, the development of AI and various biotechnologies has provided new opportunities for aging research and the drug screening. Drug screening or rejuvenation strategies based on the generalization of aging and individualized precision aging interventions will be two hot directions and trends for AI based aging interventions. These would entail using a general signature or an individualized/personalized signature (Figure 19). A step forward would be the prediction of combinatorial treatment or intervention effects, ultimately targeting aging at the whole systems level.

Summary

With the development of AI, we are pleased to see the application of AI in understanding aging mechanisms and facilitating drug screening. It is exciting to witness AI models based on 3D facial images have been used to identify longevity, deep learning-based efficacy prediction system (DLEPS) systems for drug screening of various diseases, etc. However, more experimental data are still needed to validate these studies. At the same time, as we described before, the body is a complex system, and a single facial image or other indicators cannot fully reflect the aging status of the body, and a comprehensive response of multiple indicators is required. Also due to the complexity of aging, the use of AI to screen geroprotective drugs has not been well validated. Nevertheless, with the development of omics and AI, we believe that AI will eventually become a powerful aid for research from aging mechanisms to interventions.

Novel technologies that support aging research

Advancements in technology are essential for the development of any discipline. Recently, state-of-the-art techniques have been continually developed, involving model systems, single-cell omics, bioimaging and computational analysis. These collective approaches provide vital opportunities to develop our understanding of the intricate aging process at various scales, from a single molecule to the entire organism. The following sections discuss recent methods and applications that offer new ways to study aging.

Model systems

Although large-scale cohort studies tend to provide the most compelling evidence associated with human aging, the im-

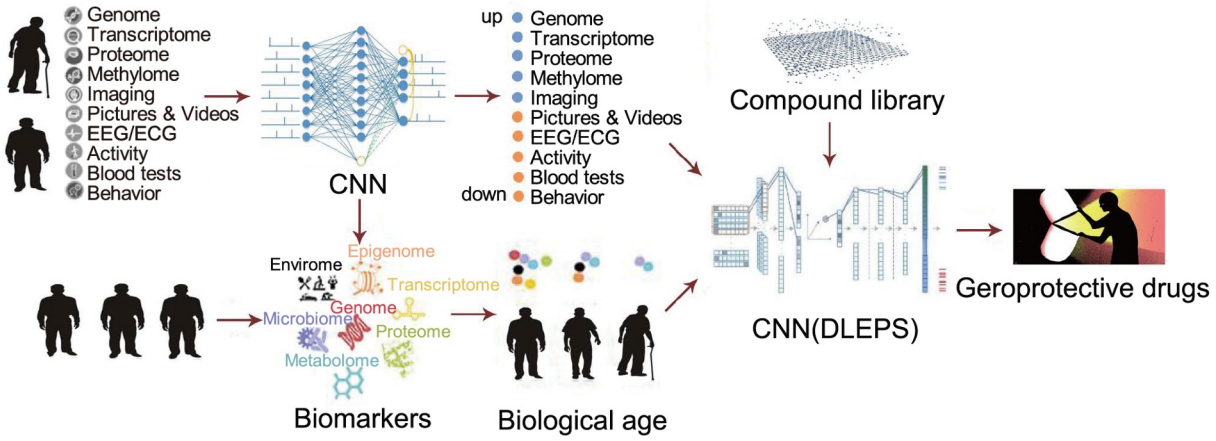

Figure 19 Two parallel and synergistic directions of AI and systems biology-based drug screening for aging intervention. Apply AI technology, such as convolutional neural network (CNN) or DLEPS, to find universal aging characteristics based on various omics data to screen geroprotective drugs. Meanwhile developing quantitative omics data based aging clocks and signatures, and use them to slow down or reverse the biological age in general or for a specific individual. Ultimately targeting aging at various level together delays or reverses human aging.

plementation difficulty and inflexibility of cohort research limit its usage. A model system that is suitable for experimental iteration and can effectively simulate aging in humans is critical for studying aging mechanisms and evaluating intervention strategies.

To this end, models of premature aging and age-related diseases are valuable tools to advance aging research. For instance, iPSCs from premature aging patients, combined with differentiation techniques and tissue engineering, serve as promising models to investigate senescence and aging *in vitro* (Atchison et al., 2020; Feng et al., 2022). However, due to the diversity of genetic backgrounds and genome instability potentially caused by reprogramming, its application is limited (Soldner and Jaenisch, 2012). The generation of unbiased embryonic stem cell models mimicking premature aging diseases, such as WS and HGPS, through gene editing, enables more stable recapitulation of premature aging cellular phenotypes, based on which several aging-associated factors have been identified (Hu et al., 2020; Liang et al., 2021; Wang et al., 2021d; Wu et al., 2018; Zhang et al., 2015b). In addition, cell models of age-related diseases, such as neurodegenerative diseases, will further broaden our understanding of aging in pathological circumstances (Kikuchi et al., 2017; Penney et al., 2020).

However, cell models are limited to simulating homogeneous systems that are sensitive to genetic mutagenesis or exogeneous stimuli. To investigate aging *in vivo*, animal models are a better choice. Physiologically aging animals and animal models of disease and accelerating aging are also valuable for advancing both basic and translational research in aging biology. With the development of genome-editing technologies, animals including rodents, rabbits, and pigs are used to simulate the characteristics of premature aging and age-related diseases (Liu et al., 2018d; Xie et al., 2019; Xu et al., 2019; Yan et al., 2018). Moreover, non-human primates (NHPs), which are evolutionarily and physiologically closer to humans, offer an ideal model to mimic human aging. A recent study has reported the first NHP model of HGPS, which exhibits similar pathological features of HGPS patients, including the presence of progerin, growth retardation, bone alterations, and vascular abnormalities (Wang et al., 2020a). Depletion of SIRT6 in NHPs also compromises neuronal differentiation, which can be simulated in the human neural progenitor cell differentiation system (Zhang et al., 2018c). BMAL1 deficiency in NHP leads to accelerated cellular senescence *in vitro*, consistent with the phenotypes of *BMAL1*$^{-/-}$ MSCs (Liang et al., 2022). Furthermore, a combination of animal and cell models may help evaluate the therapeutic potential of stem cell transplantation in age-related diseases (di Domenico et al., 2019; Sharma et al., 2019).

In addition to cell and animal models, the organoid system provides another flexible means to study the aging process, and offers a heterogeneous setting. Human organoid systems across various tissues, including those within the brain, lung, heart, and intestine, are well established (Lukonin et al., 2020; Mansour et al., 2018; Salahudeen et al., 2020; Song et al., 2022; Zhang et al., 2022a). The range of organoids enables us to study tissue heterogeneity in aging and age-related diseases. For example, cerebral organoids derived from the iPSC of AD patients exhibit relevant key pathological features including Aβ and Tau pathologies, synapse loss, and mitochondrial dysfunction (Venkataraman et al., 2022).

Single-cell omics technologies

As demonstrated in the previous sections of this review, aging is a complicated and heterogeneous process that involves progressive changes occurring at different levels. Despite efforts to decode complex multiomics characteristics of aging overall, aging is asynchronous among different in-

dividuals, tissues, and cell types, which requires a greater resolution to dissect the heterogeneity across biological contexts. Single-cell multiomics techniques provide an unprecedented opportunity to study the aging process. To date, single-cell RNA sequencing is widely applied in aging research and helps us reveal aging-related effects across tissues and species (Almanzar et al., 2020; Ma et al., 2020). In addition, single-cell genomic analysis reveals the accumulated mutations in neurons across the human lifespan (Lodato et al., 2018). Epigenetic profiling including DNA methylation and chromatin modification at the single-cell level also helps to investigate cell-type-specific aging-related epigenetic alterations (Cheung et al., 2018). Although single-cell proteomics and metabolomics analysis have not been applied in aging-related studies, they are expected to offer a more direct and in-depth mechanistic understanding of the aging process (Hartmann et al., 2021; Vistain and Tay, 2021).

In addition to monoomics single-cell analysis, current advanced single-cell multiomics technologies allow us to obtain multilayered information from the same cell. Although single-cell multiomics has not been explored in aging research, there has been some progress toward this effort. A critical step in single-cell multiomics analysis is to map molecular information to corresponding cell types, which are typically distinguished based on marker gene expression. Existing methods often combine single-cell transcriptomics with other layers of omics. Based on previous single-cell genomics analysis (Zong et al., 2012), parallel sequencing of the single-cell transcriptome and genome is completed. For example, TARGET-seq can simultaneously analyze single-cell DNA mutations and an unbiased transcriptome with a throughput of more than 4,000 cells per experiment (Rodriguez-Meira et al., 2019).

Imaging-based techniques

Compared to other methodologies, imaging-based techniques offer the advantage of preserving spatiotemporal information. In addition to the conventional immunohistochemical analysis on fixed tissues, here we review some new imaging-based methods related to aging research, including *in vivo* detection of senescent cells and spatial transcriptomics (ST) analysis.

Cellular senescence is implicated in various aging-related outcomes and therapy that targets senescent cells may mitigate physiological decline that accompanies aging. Meanwhile, cellular senescence is a complex phenomenon that requires further investigation to delineate how it occurs and is established *in vivo*, using imaging and other techniques. Recently, the primary imaging method used to detect senescent cells *in vivo* is to introduce probes for live tracking. Probes targeting β-galactosidase, a general biomarker of cellular senescence, are manipulated to visualize senescent cells. Several versions of these probes, such as AHGa, HeckGal, and DCM-βgal, increase detection sensitivity and can detect senescent cells under both normal and tumor conditions (Gu et al., 2016; Lozano-Torres et al., 2017; Lozano-Torres et al., 2021). However, β-galactosidase-based probes can also inaccurately detect non-specific induction in response to either senescence or other stress responses (Yang and Hu, 2005). Therefore, other methods are required to achieve more accurate senescent cell identification. For example, a recent study has introduced an approach using molecularly imprinted nanoparticles to target senescence-specific cell surface protein B2M to detect and remove senescent cells (Ekpenyong-Akiba et al., 2019), pinpointing membrane proteins as potential targets for accurately labeling senescent cells.

To examine spatiotemporal aging-related alterations with enhanced resolution, ST analysis is one way to integrate imaging results with gene expression data. ST analysis approaches can be divided into three categories: next generation sequencing (NGS)-based, *in situ* sequencing (ISS)-based, and *in situ* hybridization (ISH)-based methods (Rao et al., 2021). NGS-based methods (e.g., Visium, Slide-seq, DBiT-seq, Stereo-seq) add spatial barcodes before preparing the sequencing library. This allows mapping of gene expression information to spatial locations after sequencing (Chen et al., 2022; Liu et al., 2020b; Rodriques et al., 2019). ISS-based methods (e.g., STARMap, Barseq, ExSeq) also measure gene expression through sequencing, however RNA is directly sequenced within tissues to achieve ST spectrum analysis (Alon et al., 2021; Chen et al., 2019; Wang et al., 2018b). By hybridizing a fluorescent probe to the target RNA, ISH-based approaches (e.g., MERFISH, seqFISH+) quantify gene expression levels using fluorescent signals (Eng et al., 2019; Moffitt et al., 2018). ST analysis has been used to study aging and aging-related diseases in tissues including the brain, muscle, synovium, and joints (Chen et al., 2020b; Hardt et al., 2021; Perez et al., 2021; Vickovic et al., 2022). Since aging is characterized by high spatial heterogeneity, it is promising that combining ST techniques with other data sources (such as single-cell sequencing data) will provide an extensive and comprehensive understanding of the mechanisms that underlie aging.

Computational methods

Due to the development of advanced tools, different forms of massive datasets are regularly being generated. The wide application of these methods, especially omics technologies, has led to the large-scale profiling of aging-related molecules. Recent advances in computational tools and integrative databases of omics data further provide opportunities to gain new insights into aging biology (Kang et al., 2021; Liu et al., 2021). In combination with machine learning algorithms, the rich resources developed from multiomics data may help identify potential biomarkers and

targets for intervention. For example, a recent study described changes of chromatin state during stem cell aging using the ChromHMM model, and determined epigenetic driving factors of cryptic transcription via the integrative analysis of transcriptomic data (McCauley et al., 2021). Distinct stages of human epidermal tissue aging are also identified using network fusion based on transcriptome and methylome analysis (Holzscheck et al., 2020). In addition, a graphical random forest model was used to comprehensively analyze the multiomics and phenotypic data from the TwisUK cohort, linking various comorbidities of age-related diseases with molecular features (Zierer et al., 2016). These studies demonstrate the capacity to interpret molecular complexity using multiomics data and provide a novel understanding of aging and aging-related diseases.

The benefits of greater resolution include unveiling cell-type-specific molecular changes throughout aging, which presents single-cell multiomics data analysis as a more powerful tool to study aging biology. Several tools are now available to facilitate the integration of various single-cell multiomics datasets, including RNA expression and DNA methylation (MOFA+, LIGER, MATCHER) (Argelaguet et al., 2018; Welch et al., 2017; Welch et al., 2019), RNA expression and DNA copy number (Clonealign) (Campbell et al., 2019), and RNA expression and chromatin accessibility (Seurat, MATCHER) (Hao et al., 2021; Welch et al., 2017). Additionally, MATCHER is able to integrate three types of single-cell data, including RNA expression, DNA methylation, and chromatin accessibility (Welch et al., 2017). Moreover, single-cell RNA-seq profiling is suitable for combining with ST analysis, which can present the spatial locations of specific cell types. To this end, single-cell RNA-seq data will be utilized to deconvolute and map cell type information in ST data and distinguish mRNA mixtures. Available analysis tools include Seurat, SPOTlight, RCTD, and CellTrek (Cable et al., 2022; Elosua-Bayes et al., 2021; Hao et al., 2021; Wei et al., 2022a). This cell type localization information allows for ligand-receptor interactions to be further inferred through algorithms such as Giotto and SpaOTsc (Cang and Nie, 2020; Dries et al., 2021). This strategy was used in an AD study to pinpoint disease-associated cellular and molecular features near amyloid plaques (Chen et al., 2020b), however studies related to physiological aging have not been conducted to date.

Summary

The main objectives of aging research include systematically revealing the mechanisms that contribute to the aging process, identifying promising biomarkers of aging, and developing therapeutic strategies for aging and aging-related diseases. Advancing the technologies offers encouraging progress in aging research, including the future establishment of an aging model, characterizing and revealing the alterations and mechanisms of aging, and integrating data across multiple modalities using computational methods (Figure 20). Despite significant progress in technology development, many of these technologies have not been applied to aging research. Additional research is required to fully establish these paradigms and apply these novel technologies.

Conclusions and perspectives

Aging has attracted curiosity and elicited imagination throughout the human history. However, it has been only 30 years since a new epoch in aging research was established after the isolation of the first long-lived strains in *C. elegans* (Klass, 1983). Nowadays, studies in the aging field are exploding with the ever-expanding knowledge of the molecular and cellular bases of life and diseases, whilst subjected to scientific scrutiny. In this current review, we summarize the cutting-edge developments of Geriatrics and Gerontology, presenting the landscape of aging across multiple layers.

In the first chapter, at the cellular level, we focus on cellular senescence, a main culprit of aging, harnessing a panel of phenotypes from various aspects to reveal underlying molecular alterations and mechanisms. In addition, cellular senescence bridges aging and cancer, for which aging is a major risk factor but the causal relationship still remains elusive (Campisi, 2004; Campisi, 2013). On one hand, cellular senescence constitutes a potent, cell autonomous anti-cancer mechanism *in vivo* of higher eukaryotes; on the other hand, cellular senescence accumulating with age may evoke intrinsic reprogramming of stem cells and contribute to tumor-promoting microenvironment through SASP and inflammaging. Amongst all cell types, stem cell aging is of particular interest, as their exhaustion and dysfunction impair tissue function and regeneration capacity and lead to age-associated disorders, driving impacts way up to organismal aging.

Indeed, aging is manifested as a multisystemic deterioration throughout the body that leads to declining tissue and organ functions. What we have learned about cellular aging from the first chapter are also reflected at the tissue level; and moreover, in this aging community of cells, they are affected by each other in the same tissue through the microenvironment, or even across tissues by systemic factors. In the second chapter, we summarize aging-associated changes that occur in various tissues and organs, including those in the circulatory system, hematopoietic and immune system, nervous system, musculoskeletal system, reproductive system, digestive system as well as the microbiota therein. Their commonalities and tissue-specific characteristics were discussed. Collectively, understanding mechanisms and identifying targets of tissue/organ aging open vistas to therapeutic interventions for alleviating aging and age-associated disorders.

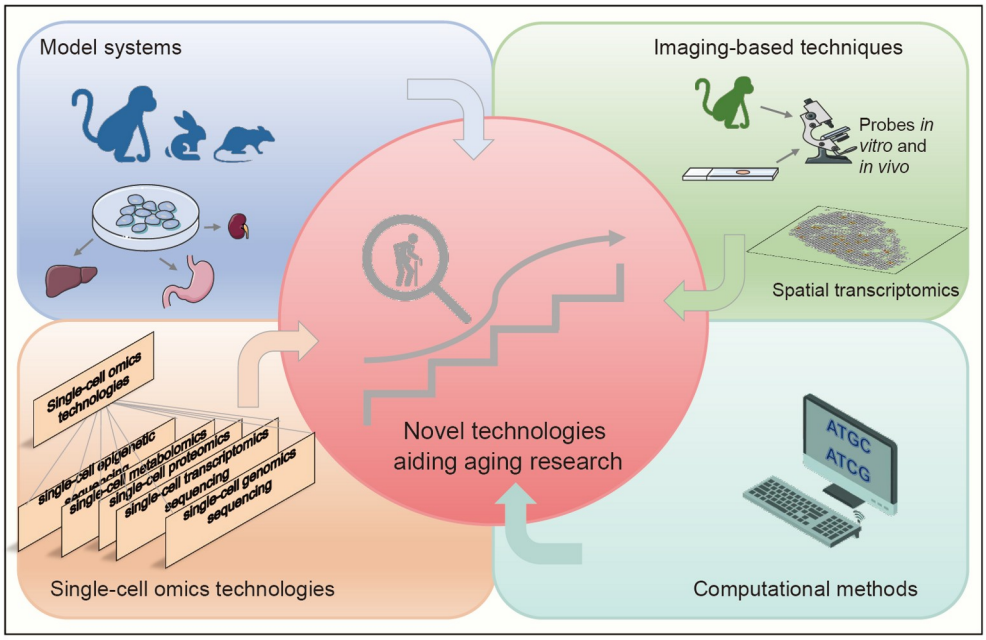

Figure 20 Four areas of novel technologies supporting aging research. The four aspects include model systems from cell and animal models to human organoids; imaging-based techniques to visualize senescent cells *in vivo* and conduct ST analysis; single-cell omics technologies to simultaneously obtain cell-type-specific multi-omics information; and computational methods for the integration of multimodal data and analysis of ST datasets.

Finally, in the third chapter, we review geroprotective approaches in the hope to rewind the biological clock to a youthful state. This can be achieved by targeting key pro-/reverse-aging factors to rejuvenate aged cells, by eliminating senescent cells, or by transplanting genetically-modified stem cells. The rejuvenating effect can be local or systematic. Sophisticated strategies have been developed to deliver it through gene therapy, antibody- or small molecules-based drugs (Partridge et al., 2020). In recent years, artificial intelligence (AI) came to the assistance of drug discovery, as well as new model systems, single-cell omics technologies, etc. (Wiley and Campisi, 2021). These state-of-the-art techniques will speed up the bench-to-bedside translation of discoveries in aging research to human therapies and clinical applications.

We are now entering an inspiring era of aging research. According to new scientific findings summarized here and in other equivalent publications, this era now offers unprecedented hope for extending human healthspan: preventing, delaying or, even in certain cases, reversing many of the signs of aging (Furman et al., 2019). Whether this era promises to extend the longest human lifespan still remains an open question. However, what is clear is that after 30 years of fundamental research linking specific genes to aging, although many aspects still await further investigation, such as the interplay between metabolism and systemic aging, a solid foundation has been established, and clinical trials for interventions that target the aging process are being initiated. Although we may encounter considerable difficulties in applying this research to human, the potential rewards in healthy aging far outweigh the risks. Complicated as aging is, it is encouraging that new model systems and emerging tools of modern biology, including single-cell omics, systems biology and AI are making it easier to gain insights into complex interactions, which will no doubt have a significant impact on the development of more geroprotective strategies. In fact, the more progress we make in healthy aging, the greater the value of further improvements.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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