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Tomoki Shiozawa · Hiromi Hirata ·
Takashi Inoue · Dominika Kanikowska ·
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Gerontology as an Interdisciplinary Science

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Gerontology as an Interdisciplinary Science

Springer

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Preface

The society is experiencing significant demographic transformations characterized by phenomena including aging and increased longevity. The rising population of those reaching the age of 100 and the provision of support by society and corporations for their well-being have emerged as crucial concerns. Japan's trajectory toward a super-aging society is particularly striking. Its population has reached an aging rate of 29.1% by 2022, accompanied by an unprecedented average life expectancy of 81.47 years for males and 87.57 years for females. Gerontology has garnered considerable attention as a field endeavoring to illuminate this pressing issue and to reconceptualize the elderly as a novel sociocultural resource.

Deriving its name from the longevity research conducted by microbiologist Ilya Metchnikoff (recipient of the Nobel Prize in Physiology or Medicine) at the Pasteur Institute in France in 1903, gerontology experienced significant advancements primarily in the United States after the 1930s. Meanwhile in Japan, academic societies, such as the Japan Gerontological Society, have been actively engaged in this domain since the 1960s. In recent years, gerontology has been recognized as an interdisciplinary research field. It investigates the multifaceted aspects of aging that encompass biological, social-scientific, and psychological dimensions, thereby making valuable contributions to societies grappling with the challenges posed by super-aging.

Considering these backgrounds, we are delighted to present "Gerontology as an Interdisciplinary Science," a collaborative publication between the Japanese Society for Hygiene, Poznan Medical University, Poznan, Poland, and the Institute of Gerontology at Aoyama Gakuin University, Tokyo, Japan. This book features contributions from 18 authors, including esteemed university professors and experts in the field. It is organized into three sections, each featuring six authors, collectively encompassing a broad spectrum of gerontology-related topics. The papers of the initial section, titled "Environmental Health and Gerontology," investigate the intricate interplay between environmental factors and the aging process. The subsequent section, "Aging Societies and Gerontology," delves into the multifaceted challenges and opportunities arising from an increasingly aging population. The last section, "Preventive Medicine and Gerontology," delves into the exploration of strategies

aimed at averting or postponing age-related ailments and disorders. Each of these sections offers user-friendly algorithms and key insights that facilitate comprehension. By amalgamating social science research with preventive and environmental medicine investigations, a synergistic interdisciplinary framework emerges. This engenders novel academic disciplines and propels groundbreaking innovations.

The papers featured in this book encompass a diverse range of subjects, spanning medical and physiological investigations concerning both humans and laboratory animals, as well as disciplines such as engineering, economics, and ethics. These contributions offer invaluable insights into the intricate and multifaceted dimensions of aging and its far-reaching impact on individuals, families, and societies at large. With its comprehensive coverage, this book serves as an indispensable resource for scholars specializing in environmental and occupational health worldwide, as well as for both aspiring and established researchers, students, and field practitioners; a thorough understanding of the caregiving, well-being, and health promotion aspects pertaining to older adults can be elucidated. Additionally, this book holds significance for researchers working across various fields, including robot operation interfaces and the analysis of emotional responses through biosignals among the elderly. Moreover, it serves as a valuable source of knowledge for creators and technical personnel involved in biosignal data science and database management, leading to a further step in comprehending the unique needs and characteristics of the elderly population.

Lastly, we wish to extend our heartfelt gratitude to the production editors and the editorial team at Springer Nature for their invaluable assistance in bringing this book to fruition. Their steadfast patience and unwavering commitment to professionalism have been instrumental throughout the entire editorial journey. Without their dedicated support, the publication of this book would not have been achievable.

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The original version of the book has been revised. Abstracts and keywords has been updated for all chapters. The correction to this book is available at https://doi.org/10.1007/978-981-97-2712-4_19

Contents

Part I
Environmental Health and Gerontology

Chapter 1
Aging and Senescence Studies in Human and Zebrafish

Hiromi Hirata, Tsuyoshi Tezuka, and Kota Ujibe

Abstract Everyone experiences aging in his/her life. Since aging is a deteriorative process toward the end of life, most people desire longevity. However, we do not know how we can extend our life span. We do not even know what the actual cause of aging is. Senescence, which is a cellular process of aging, is a hallmark of animal aging. In this review, we introduce many papers that unveiled the process and cause of aging and senescence along with aging-related diseases in humans. We also discuss fish models to study aging and senescence.

Keywords Aging · Senescence · Cell cycle · DNA damage · Telomere · Disease · Zebrafish

Abbreviations

Aβ	Amyloid-β
CDK	Cyclin-dependent kinase
cDNA	Complementary DNA
DNA	Deoxyribonucleic acid
GFP	Green fluorescent protein
GWAS	Genome-wide associate studies
mRNA	Messenger ribonucleic acid

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PET	Positron emission tomography
RFP	Red fluorescent protein
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT-PCR	Reverse transcription polymerase chain reaction
SAβGal	Senescence-associated beta-galactosidase
TILLING	Targeting-induced local lesion in genome

1.1 Introduction

Many Western countries and East Asian countries undergo population aging. Indeed, the percentage of people aged 65 and over in Japan was 17% in 2000 and 28% in 2020 and will reach 35% in 2040 and 38% in 2060 (https://www8.cao.go.jp/kourei/whitepaper/index-w.html). The increase in the number of elderly people has been accelerated by reduced birth rate and extended longevity, the latter being enhanced by the recent significant advances in life science to study aging and senescence. Aging is the gradual process of becoming old that accompanies a progressive decline of physiological integrity and function. The irreversible deterioration of organs and tissues is followed by various aging-related pathology and diseases such as high blood pressure, arteriosclerosis, rheumatism, diabetes, cancer, infarction, stroke, macular degeneration, Alzheimer's disease, Parkinson's disease and many others. Senescence is another term for aging, mostly used at the cellular level. Senescent cells keep normal homeostasis and are functionally active but show cell cycle arrest and cease proliferation. By searching for scientific papers in PubMed, which is a free search engine to access publications on life science and biomedical topics, I could find several papers harboring either "aging"or "senescence"as a keyword (Fig. 1.1). The number of papers searchable by either of these keywords significantly increased in the twenty-first century, revealing that aging and senescence have been expansively explored as scientific interests.

The two major questions of aging and senescence in humans are "How do we age?" and "Why do we age?" The former is an issue of hallmarks of aging, including altered homeostasis, physiological symptoms and pathology. The latter is a mechanistic issue toward understanding the causes of aging. It is now known that senescence contributes to aging. Many genetic mutations that accelerate cellular senescence and/or animal aging have provided molecular insights into aging, leading to understanding normal homeostasis and physiological integrity that avoids senescence and aging. This review focuses on the molecular mechanisms and theories of senescence and aging in humans and mice. I also raise an emerging model animal, a zebrafish, to extensively study senescence and aging.

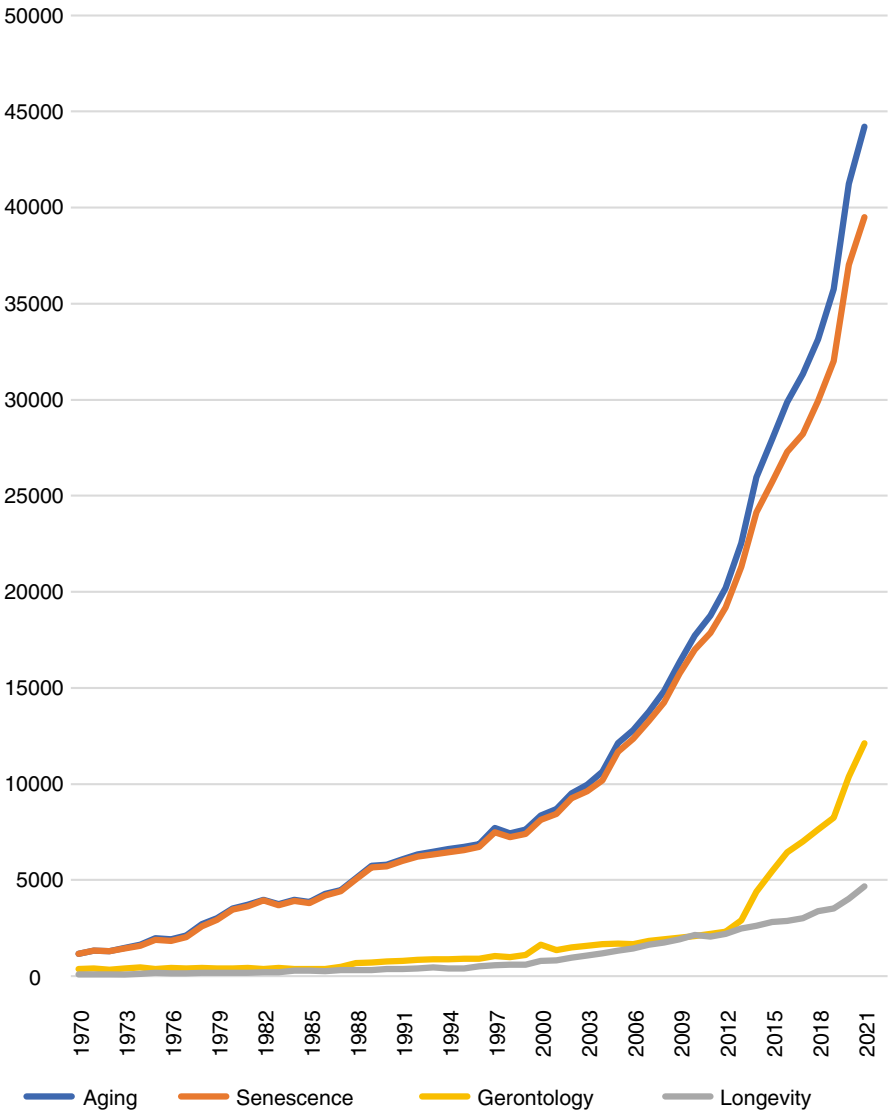

Fig. 1.1 Number of publications related to aging. A number of aging-related papers were investigated by PubMed search from 1971 to 2022

1.2 Senescence

Cellular senescence was first described in cultured human cells by Hayflick and Moorhead [1]. Normal human fetal fibroblasts cease to divide after 50 times of passage. They suggested that cells repeat cell division up to certain times, referred to as the Hayflick limit, but then enter the phase of irreversible growth arrest. The key

question of this cellular senescence is how cells count the number of their cell divisions. In other words, what molecules accumulate or are depleted in the senescent cells? There are three major molecules known to regulate the cellular senescence.

1.2.1 Telomere

Telomere is a DNA-protein structure comprising repetitive hexanucleotide (TTAGGG) sequences [2, 3]. Telomere-associated shelterin proteins are located at the ends of chromosomes [4]. Although this terminal DNA region is maintained by the telomerase reverse transcriptase enzyme complex during the DNA replication process, the actual length of the telomere progressively shortens with each cell cycle division. In fact, the length of telomere shortens from 11 kb at birth to 4 kb at old [5, 6]. The shortening of telomere triggers cellular senescence and unwanted chromosome fusions [7, 8]. In agreement with these observations, cancer cells that divide continuously maintain high levels of telomerase activity [9]. Mutations in the telomerase reverse transcriptase gene cause a premature aging syndrome, dyskeratosis congenita [6]. Similarly, mutations of telomeric DNA-binding protein 1 (TRF1)-interacting nuclear factor 2 cause the same disease [10]. Mice deficient in the telomerase reverse transcriptase gene showed a short length of telomeric DNA and a short life span [11]. Conversely, mice that overexpress telomerase reverse transcriptase showed an increase in lifespan [12]. Telomere shortening is one of the major factors for cellular senescence and aging.

1.2.2 Oxidative Stress

Reactive oxygen species (ROS) is another major factor in the cellular senescence [13]. ROS is a subset of highly reactive molecules comprising superoxide anion (O_2^-), hydroxyl radical (OH^-), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2). In some cases, nitric oxide (NO^-) and peroxynitrite ($ONOO^-$) are also included as ROS. Superoxide anion, which is produced by a decrease in electron transfer in mitochondrial respiration, is converted to hydrogen peroxide by superoxide dismutase (SOD). Hydrogen peroxide is normally degraded into oxygen and water by catalase. However, hydroxyl radical and singlet oxygen also arise from hydrogen peroxide at low frequencies in all cells. ROS causes unwanted oxidative reactions, including DNA base oxidation, lipid peroxidation and protein carbonylation. These damaged molecules are created continuously and repaired in normal homeostasis. In low levels of ROS, mild oxidative stress tonically activates the repair system and even contributes to lengthening the lifespans of organisms [14]. ROS is also used as a second messenger to promote cell cycle progression and cell fate decision [15, 16]. In high levels of ROS, on the other hand, severe oxidative stress causes the accumulation of DNA damage and damaged macromolecules that in turn alter gene

expression and further increase ROS, especially in mitochondria, where ROS is generated. This unfortunate positive feedback changes metabolism, epigenetic regulation and homeostatic integrity at multiple steps and induces cell cycle arrest to enter the senescent state. Oxidative stress also stimulate telomere shortening [17].

1.2.3 DNA Damage

The shortening of telomere is recognized as DNA damage. In addition to the telomere shortening and ROS-mediated DNA base oxidation, spontaneous DNA mutation generated by replication errors is a major cause of DNA damage. Cells have several repair systems to cope with different types of DNA damage. The error of DNA replication and ROS-mediated base oxidation generate a mismatch of base pairs. This mismatch is repaired by either base excision repair, mismatch repair, translesion synthesis or break-induced recombination [18]. ROS also induces a single-strand break of DNA, which is repaired by a single-strand break repair. Ionizing radiation creates a double-strand break, which is the most severe damage in DNA. Double-strand break is repairable by either homologous recombination, non-homologous end joining or single-strand annealing [19]. Upon the double-strand breaks, unusual terminal ends of DNA are recognized by ataxia-telangiectasia mutated (ATM) and ataxia-telangiectasia-and-RAD3-related (ATR) proteins. ATM phosphorylates p53, which promotes stabilization and nuclear localization of p53. Activated p53 functions as a transcription factor and induces the expression of a cell cycle inhibitor gene $p21^{Cip}$. This transcriptional regulation causes cell cycle arrest to gain time to repair the damages. However, if there is too much DNA damage, p53 activation goes over the threshold, triggering the activation of the apoptosis pathway. This system eliminates DNA damage. If the cells with a number of DNA damages, including mismatch of base pairs, did not undergo apoptosis and enter the cell division, the mutation is to be fixed in DNA. Since the accumulation of DNA mutations induces cancer, DNA damage is linked to carcinogenesis. To avoid the formation of cancer cells, cells with a lot of DNA damage enter the senescent status and are sometimes removed from the tissues. Thus, the accumulation of DNA damage is a factor in cellular senescence and aging.

Phosphorylation of H2AX, a histone variant, is a marker of DNA damage. Histon octamers bind to DNA to form chromosomes and are composed of two H2A/H2B dimers, two H3 and two H4 proteins. H2AX is a minor variant of H2A [20]. In response to the double-strand breaks of DNA, the serine-139 residue at the C-terminus of H2AX is phosphorylated by ATM near the damaged sites. This phosphorylated form of H2AX is referred to as γH2AX. The γH2AX contributes to recruiting various DNA repair systems to the lesion sites. In fact, DNA damage is not repaired in H2AX-deficient cells, suggesting the essential role of γH2AX in the recognition and repair of DNA damage [21, 22]. The γH2AX protein also activates cell cycle inhibitors to induce cell cycle arrest. The amounts of γH2AX increase in senescent cells, probably because aged cells have many genomic lesions. In

addition, DNA damages that remain unrepaired accumulate in senescent cells [23]. The increase of γH2AX is also seen in tissues of aged animals [24, 25], further supporting the notion that γH2AX is a marker of DNA damage as well as cellular senescence and aging.

1.2.4 Cell Cycle Inhibitors

Cell cycle inhibitors such as $p16^{INK4a}$, $p14^{ARF}$ ($p19^{ARF}$ in mice), $p21^{Cip}$ and $p15^{INK4b}$ interfere with cyclin-CDK function and cause cell cycle arrest [26]. These proteins are known as senescence markers, as they are expressed at high levels in senescent cells. The p16INK4a/p14ARF gene locus encodes p16INK4a and p14ARF proteins in a complicated manner. The transcripts of these two products are generated from different exon 1 (exon 1a and 1b) using different promoters. But they share the same exons 2 and 3. Interestingly, two transcripts use different reading frames for exons 2 and 3. Thus, $p16^{INK4a}$ and $p14^{ARF}$ do not have any amino acid homology and function in different manners. The $p16^{INK4a}$ binds to cyclin-dependent kinases CDK4 and CDK6, inhibiting CyclinD/CDK4 and Cyclin D/CDK6 function. Since these two cyclin-CDK complexes work for cell cycle progression in the G1 phase, $p16^{INK4a}$ causes cell cycle arrest at the G1 phase. The $p14^{ARF}$ binds to and inhibits MDM2, which binds to p53 and promotes ubiquitination and proteasome-dependent degradation of p53. Thus, $p14^{ARF}$ activates p53 that leads to the expression of $p21^{Cip}$, which binds to CDK4, CDK6 and CDK2 and inhibits CyclinD/CDK4, Cyclin D/CDK6 and CyclinE/CDK2 function, ceasing the cell cycle progression in G1 phase. Likewise, the $p15^{INK4b}$ protein binds to CDK4 and CDK6, contributing to the cell cycle arrest. The expression of the above cell cycle inhibitors is maintained at low levels in normal cells and tissues in young animals but becomes upregulated during continuous culture passages and animal aging [27]. Although numerous transcriptional activation, repression, and epigenetic regulations of these genes have been reported in the context of cancer development, transcriptional regulation of these genes in senescence remains to be solved [28]. Among these cell cycle inhibitors, $p16^{INK4a}$ has been suggested to be most relevant to senescence. The expression of $p16^{INK4a}$ increases gradually during development, keeping the low level in adulthood, but becomes dramatically upregulated in the old animals [29]. This significant increase in $p16^{INK4a}$ expression in aged animals suggests that $p16^{INK4a}$ is the most prominent marker of aging and senescence [30].

1.2.5 Senescence-Associated β-Galactosidase

Senescence-associated beta-galactosidase (SAβGal) is a classic marker of cellular senescence originally reported in 1995 [31]. Generally, β-galactosidase activity is assayed by the hydrolysis of 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal)

into monosaccharides. This product forms dark blue precipitates after oxidation, enabling the visualization of β-galactosidase activity as the blue color. The SAβGal, which was detectable specifically at pH 6 as blue labeling, was upregulated in senescent cells and aged tissues but not in young cells, terminally differentiated cells and immortal cells as well as in young tissues. SAβGal is a lysosomal β-galactosidase encoded by *galactosidase beta 1 (GLB1)* gene [32]. Since SAβGal is an easy assay for detecting the distribution of aged cells, this evaluation method has been widely used for cellular senescence and animal aging research.

1.3 Aging

Aging is an intrinsic functional decline of homeostasis and physiological integrity [33]. What is the direct cause of aging remains a challenging question. Undoubtedly, cellular senescence is one of the major direct causes of aging. However, several genes induce senescence and harbor anti-senescent functions such as apoptosis and quiescence. This paradoxical function makes it difficult to clarify the relationship between senescence and aging. There are a number of aging-related diseases in humans, as follows.

1.3.1 Cancer

Cancer is characterized by abnormal cell proliferation, invasion from the tissue and metastasis to the other tissues. Cancer is the second leading cause of death globally. Indeed, 27% of the cause of death in Japan was cancer in 2021. Since cancer is induced by DNA mutations and since tumor suppressor genes such as p53 and $p16^{INK4a}$/$p14^{ARF}$ are involved in the induction of cellular senescence, senescence is likely a tumor suppressor mechanism and thus cancer is a failure of the cellular senescence [34, 35]. Similarly, the increase of senescent cells with age increases cancer incidence. In agreement with this notion, if senescent cells are removed from the aged tissues, the onset of tumor formation is delayed [36]. This strategy is potentially applicable to cancer elimination, which is referred to as senolytic therapy [37].

1.3.2 Cardiovascular Disease

Cardiovascular diseases are disorders of the heart and blood vessels and are the leading cause of death globally. The blood vessels of the cardiac muscles, brain and arms/legs are affected by coronary heart disease, cerebrovascular disease and peripheral arterial disease, respectively. The risk of arteriosclerosis of any artery blood vessels increases with age, which in turn causes rupture and infarction of

blood vessels, leading to cardiovascular disease. Macrophage cells are the primary senescent cells that show high SAβGal activities in the blood vessels [38]. Cardiomyocyte atrophy is another infarction disease that frequently occurs in old people [39].

1.3.3 Kidney Dysfunction

The glomerular filtration rate decreases and urine albumin increases with age through the development of nephrosclerosis and nephron atrophy. Cellular senescence marked by increased p53 and $p16^{INK4a}$ expression is linked with these kidney aging [40–42].

1.3.4 Diabetes

Diabetes is a hyperglycemia disease, which is also evident in the high glucose in the urine [43]. Type 1 diabetes occurs by the elimination of the insulin-producing Langerhans β cells in the pancreas. Insulin is a hormone that reduces blood sugar levels. In many cases of type 1 diabetes, the immune system attacks and eliminates the β cells. Type 2 diabetes occurs due to the insufficient use of insulin. More than 90% of the patients who have type 2 diabetes are deteriorated by excess body weight and physical inactivity along with age. Genome-wide associate studies (GWAS) have revealed that SNPs in the $p16^{INK4a}$/$p14^{ARF}$ locus are linked to the risk of type 2 diabetes [44, 45]. This evidence suggests that cellular senescence causes diabetes as an age-associated disease.

1.3.5 Cirrhosis

Cirrhosis is a fibrosis of the liver caused by long-term liver damage, including nonalcoholic fatty liver disease, hepatitis and excess alcohol consumption [46]. The senescence of hepatocytes is also linked to nonalcoholic liver disorders [47, 48]. Senolytic elimination of senescent hepatocytes improved liver condition and suppressed fat accumulation [49].

1.3.6 Sarcopenia

Sarcopenia is a muscle atrophy that deteriorates with age and physical inactivity [50]. Skeletal muscle cells can be classified into fast-twitch and slow-twitch muscle fibers. While fast-twitch muscles make sudden and powerful movements in sprints,

slow-twitch muscles enable sustained movements in the marathon. In young individuals, the decrease of muscle is compensated by myogenesis, in which muscle stem cells, which are often referred to as muscle satellite cells, differentiate into muscle fibers [51]. This regeneration potential for both fast-twitch fibers and slow-twitch fibers declines with age, along with the accumulation of $p16^{INK4a}$ in muscle satellite cells [52]. In sarcopenia, the reduction of muscle fibers occurs predominantly in fast-twitch muscles. Interestingly, senolytic elimination of senescent muscle satellite cells enhanced myogenesis [53].

1.3.7 Osteoporosis

Bones are continuously destructed by osteoclasts and built by osteoblasts, and thus, they are kept remodeling for a lifetime [54]. But as we get older, we lose more bone than we build due to the dysregulation of bone homeostasis. Osteoporosis is the reduction of bone density with aging that causes bone fractures. These osteoporotic fractures restrict movements and then promote sarcopenia, thus triggering positive feedback toward bedridden or immotile life. In aged bones, senescent osteoblasts that express high levels of $p16^{INK4a}$, $p21^{Cip}$ and p53 and show cell cycle arrest were found [55]. Targeted removal of senescent osteoblasts mitigated osteoporosis symptoms in aged mice [56].

1.3.8 Neurodegenerative Diseases

Neurodegenerative diseases are disorders caused by progressive loss of neuronal cells [57]. The major causes of neuronal cell death are oxidative stress, inflammation, neurotoxins, insufficient neuronal excitability, poor nutrition, mitochondrial dysfunction and accumulation of abnormal proteins, triggering the apoptosis pathways. In the world. About 50 million people currently suffer from neurodegenerative diseases, and the symptoms deteriorate with age. It is suggested that the number of neurodegenerative disease patients will increase to 115 million by 2050, along with the increase in our lifespan [58].

Alzheimer's disease is the most common dementia characterized by progressive loss of brain functions such as memory, language, thinking, mental control and consciousness [59]. In patients, the shrinkage of the brain can be observed by positron emission tomography (PET), especially in the hippocampal area, which is a brain region for memory storage and retrieval. Abnormal aggregation of the amyloid-β (Aβ) peptides and tau proteins in the brain is the major pathological condition and one of the direct causes of Alzheimer's disease. However, the actual cause for most patients is unclear. About 1–2% of Alzheimer's disease are inherited in an autosomal dominant manner [60]. Mutations in either Aβ precursor protein, presenilin 1 or presenilin 2, the latter two promoting the aggregation of Aβ, are responsible for the genetic cases of Alzheimer's disease [61]. Although our brain

can remove these protein aggregations, the capability of the clearance decreases with age. Thus, age-dependent accumulation of harmful proteins causes Alzheimer's disease in old people.

Parkinson's disease is a neurodegenerative disorder affecting the motor system and mental control [62]. The typical symptom of this disease is motor disability, including tremors, stiffness and slow movement. In patients, dopaminergic neurons in the substantia nigra of the midbrain are eliminated along with age, triggering the motor and mood deficits. Cytoplasmic and intranuclear accumulation of a-synuclein protein are typical features and one of the direct causes of Parkinson's disease [63]. The clearance capability of these abnormal protein aggregates decreases with age. About 5–10% of Parkinson's disease are inherited in an autosomal dominant or recessive manner. One of the causative genes is *SNCA*, encoding the α-synuclein, which forms cytotoxic aggregates that kill dopaminergic neurons [64].

Macular degeneration is another neurodegenerative disorder characterized by the loss of the macula of the retina in the eye [65]. Macula is located at the center of the retina and is necessary to collect the central vision. Macular degeneration patients gradually lose the middle part rather than the peripheral part of the vision. About 50% of macular degeneration is linked to several chromosome loci [66]. Indeed, some genes encoding for complement factor proteins are associated with the risk, revealing that inflammation is pathologically relevant for macular degeneration [67]. The accumulation of intracellular and extracellular debris such as lysosomal lipofuscin, retinal drusen and metabolic wastes generated by the inflammation deteriorates age-related macular degeneration.

Taken together, the above neurodegenerative diseases have similar pathological bases related to the toxic aggregates. First, abnormal protein aggregates inside and outside of neurons are formed. Second, the protein aggregates accumulate with age. Finally, the aggregates promote cell death in the specific neurons/neuronal tissues and develop age-related neuronal disorders.

1.4 Fish Models

Model animals have been applied for biomedical studies including aging and senescence. Among a variety of model animals, freshwater fishes such as zebrafish and medaka have been extensively used in the twenty-first century. Zebrafish became the second major model vertebrate animal next to mice. Indeed, the number of hits by PubMed search using "zebrafish" as a keyword is now larger than those using "Drosophila," "*C. elegans*" or "*Saccharomyces cerevisiae*" (Fig. 1.2).

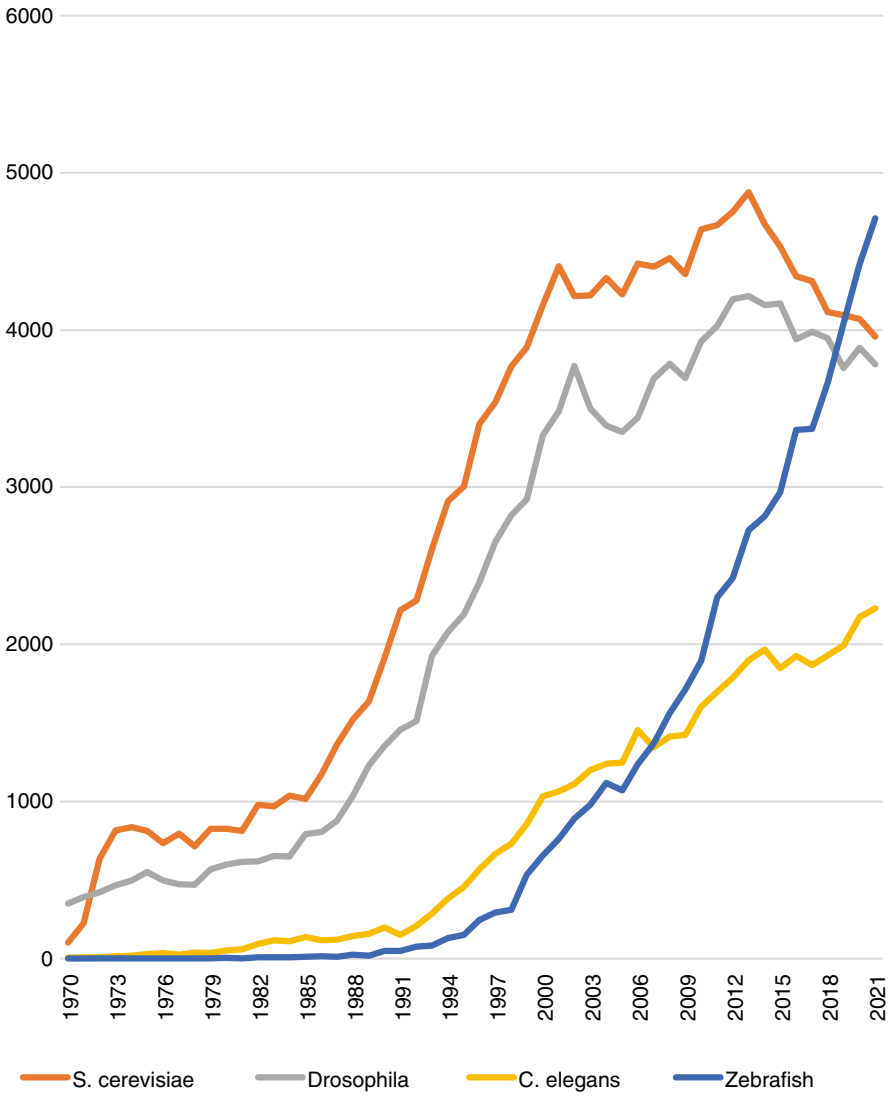

Fig. 1.2 Number of publications related to model organisms. A number of model organism-related papers were investigated by PubMed search from 1971 to 2022

1.4.1 Advantages of Zebrafish

Zebrafish (*Danio rerio*) have many advantages in biomedical research [68]. First, the husbandry of zebrafish is space- and cost-effective than that of mice. A pair of adult zebrafish can produce 200 fertilized eggs every week. They become sexually mature 3 months after fertilization. Second, the development of zebrafish takes

place externally and rapidly, with the speed of embryogenesis 80-fold faster than humans [69]. Indeed, zebrafish hatches from chorion within 3 days of development, while the human gestation period is 266 days. Third, methods of genetic manipulation have been established in zebrafish to generate mutants by forward and reverse genetics as well as transgenics by transposon system [70–82]. Several mutagenesis projects have created a number of zebrafish mutants since the 1990s [83–85]. In conjunction with the transparency during embryogenesis, tissue morphogenesis can be visualized in live zebrafish. These advantages make the zebrafish a powerful vertebrate model for studying development, disease and aging [86].

1.4.2 Senescence and Aging Studies in Zebrafish

In zebrafish, cellular senescence occurs, just as in mammals [87–89]. The increase of SAβGal activities in aged fish's skin, muscle and retinal cells has been reported [90]. Expression of cell cycle inhibitors such as $p16^{INK4a}$, $p15^{INK4b}$ and $p21^{CIP}$ accompanies the nuclear accumulation of γH2AX [91].

The lifespan of zebrafish is 3–4 years, while some fish survive over 5 years under a good husbandry condition [90, 92]. The curvature of the spine and thus hunchbacked appearance is often seen in aged zebrafish. Along with age, they also show an increase in SAβGal activities in the skin and lipofuscin, which are pigment granules in the liver. Zebrafish also show a decline in regeneration capacity and motor ability [93, 94]. Telomere-deficient zebrafish showed increased DNA damage markers such as p53 binding protein and γH2AX as well as apoptosis [95, 96]. They also exhibited accelerated degenerative phenotypes, including emaciation, infertility, inflammation and shortened half-life (0.8–1.3 years). Interestingly, most offspring obtained from a pair of telomere-deficient fish and wild-type fish showed developmental malformations. Taken together, aging in zebrafish seems to occur as in mammals.

1.4.3 Diabetes Models in Zebrafish

Excess feeding or high-fat diet can induce obesity and diabetes symptoms in zebrafish [97, 98]. Expression of a constitutive-active variant of human AKT1 with a cell membrane-anchoring myristoylation signal in skin epidermal cells caused hypertrophic skin growth and obese phenotypes at the adult stage, the latter including the increase of body weight, hyperplastic growth of adipocytes, excess accumulation of fat tissues and impaired glucose intolerance [99]. Since these phenotypes are partly ameliorated by antisense morpholino-mediated knockdown of mTOR homolog in the transgenic zebrafish, the Akt-mTOR axis plays a conserved role in the diabetes of vertebrates [100].

1.4.4 Sarcopenia Models in Zebrafish

Zebrafish have fast-twitch and slow-twitch muscle fibers. While these two types of muscle cells are mosaically distributed in human musculature, fast-twitch and slow-twitch muscle fibers are exclusively located at deep layers and superficial areas underneath the skin, respectively, in zebrafish [101]. This segregation enables easy identification of muscle cell types in zebrafish. Aged zebrafish show a reduction of muscle mass in both fast-twitch and slow-twitch muscle fibers that correlate with the deterioration of swimming performance [94]. So far, many genetic mutants that have defects in fast- and/or slow-twitch muscle development have been generated [102]. Some were established as muscular dystrophy models [103–110] and myopathy models [111–123]. However, these mutants died during embryogenesis and were not available as a sarcopenia model. Zebrafish that display adult-onset muscle atrophy are necessary to assess sarcopenia in zebrafish.

1.4.5 Neurodegenerative Diseases Models in Zebrafish

Progressive degeneration of neuronal cells has also been studied in zebrafish. Zebrafish treated with scopolamine or okadaic acids, which are antagonists of acetylcholine receptors or protein phosphatase 2A, respectively, showed behavioral and histological defects associated with Alzheimer's disease [124, 125]. Zebrafish have two amyloid peptide precursor homologs (*appa* and *appb*) and *appb*-deficient zebrafish showed impairments in locomotion and behavior [126, 127]. Expression of a pathogenic variant of amyloid peptide precursor under the *appb* promoter in zebrafish generated an Alzheimer's disease, in which behavioral deficits, beta amyloidosis and neuronal cell death are observed [128].

In zebrafish, administration of neurotoxins such as 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rotenone-induced cell death of dopaminergic neurons in the midbrain. These treatments generate models of Parkinson's disease [129–131]. These disease models show both motor deficits and non-motor behavioral impairments, which are characteristic of Parkinson's disease in humans. Genome-wide association studies in humans have identified many genes linked to Parkinson's disease. Many zebrafish models of Parkinson's disease have been generated by knockdown, knockout and transgenic expression [132–140]. For example, *park2* knockdown zebrafish showed reduced dopaminergic neurons and impaired mitochondrial respiratory functions [141, 142]. Similarly, *pink1*-deficient zebrafish displayed the loss of dopaminergic neurons and dysfunction of mitochondria [143, 144]. Other than neurodegenerative ones, there are many neurologically defective mutants in zebrafish [145–149].

1.5 Concluding Remarks

Aging and senescence are undoubtedly some of the biggest issues in life science, but they are still thoroughly unexplored. Recent advances in genome sequencing, genome editing and in vivo analyses have expanded our knowledge on the process of aging and senescence. Furthermore, animal models, including emerging zebrafish models, became available for manipulations and detailed analyses of the basis of aging and senescence as well as therapy experiments and drug screening. Future studies of these non-human studies will enable practical approaches for anti-aging.

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Conflict of Interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res. 1961;25:585–621.
2. Creighton HB, McClintock B. A correlation of cytological and genetical crossing-over in Zea Mays. Proc Natl Acad Sci U S A. 1931;17(8):492–7.
3. Revy P, Kannengiesser C, Bertuch AA. Genetics of human telomere biology disorders. Nat Rev Genet. 2023;24(2):86–108.
4. de Lange T. Shelterin-mediated telomere protection. Annu Rev Genet. 2018;52:223–47.
5. Okuda K, et al. Telomere length in the newborn. Pediatr Res. 2002;52(3):377–81.
6. Bischoff C, et al. Telomere length among the elderly and oldest-old. Twin Res Hum Genet. 2005;8(5):425–32.
7. Palm W, de Lange T. How shelterin protects mammalian telomeres. Annu Rev Genet. 2008;42:301–34.
8. Barnes PJ. Mechanisms of development of multimorbidity in the elderly. Eur Respir J. 2015;45(3):790–806.
9. Stewart SA, Weinberg RA. Telomeres: cancer to human aging. Annu Rev Cell Dev Biol. 2006;22:531–57.
10. Walne AJ, et al. TINF2 mutations result in very short telomeres: analysis of a large cohort of patients with dyskeratosis congenita and related bone marrow failure syndromes. Blood. 2008;112(9):3594–600.
11. Blasco MA, et al. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell. 1997;91(1):25–34.

12. Gonzalez-Suarez E, et al. Antagonistic effects of telomerase on cancer and aging in K5-mTert transgenic mice. Oncogene. 2005;24(13):2256–70.
13. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000;408(6809):239–47.
14. Ristow M, Schmeisser S. Extending life span by increasing oxidative stress. Free Radic Biol Med. 2011;51(2):327–36.
15. Verbon EH, Post JA, Boonstra J. The influence of reactive oxygen species on cell cycle progression in mammalian cells. Gene. 2012;511(1):1–6.
16. Kaminskyy VO, Zhivotovsky B. Free radicals in cross talk between autophagy and apoptosis. Antioxid Redox Signal. 2014;21(1):86–102.
17. Passos JF, Saretzki G, von Zglinicki T. DNA damage in telomeres and mitochondria during cellular senescence: is there a connection? Nucleic Acids Res. 2007;35(22):7505–13.
18. Clarke TL, Mostoslavsky R. DNA repair as a shared hallmark in cancer and ageing. Mol Oncol. 2022;16(18):3352–79.
19. Waterman DP, Haber JE, Smolka MB. Checkpoint responses to DNA double-strand breaks. Annu Rev Biochem. 2020;89:103–33.
20. Rogakou EP, et al. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem. 1998;273(10):5858–68.
21. Celeste A, et al. Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. Nat Cell Biol. 2003;5(7):675–9.
22. Celeste A, et al. H2AX haploinsufficiency modifies genomic stability and tumor susceptibility. Cell. 2003;114(3):371–83.
23. Sedelnikova OA, et al. Senescing human cells and ageing mice accumulate DNA lesions with unrepairable double-strand breaks. Nat Cell Biol. 2004;6(2):168–70.
24. Bakkenist CJ, et al. Disappearance of the telomere dysfunction-induced stress response in fully senescent cells. Cancer Res. 2004;64(11):3748–52.
25. d'Adda di Fagagna F, et al. A DNA damage checkpoint response in telomere-initiated senescence. Nature. 2003;426(6963):194–8.
26. Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: a changing paradigm. Nat Rev Cancer. 2009;9(3):153–66.
27. Krishnamurthy J, et al. Ink4a/Arf expression is a biomarker of aging. J Clin Invest. 2004;114(9):1299–307.
28. Sharpless NE, Sherr CJ. Forging a signature of in vivo senescence. Nat Rev Cancer. 2015;15(7):397–408.
29. Wagner KD, Wagner N. The senescence markers p16INK4A, p14ARF/p19ARF, and p21 in organ development and homeostasis. Cells. 2022;11(12):1966.
30. Gonzalez-Gualda E, et al. A guide to assessing cellular senescence in vitro and in vivo. FEBS J. 2021;288(1):56–80.
31. Dimri GP, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci U S A. 1995;92(20):9363–7.
32. Lee BY, et al. Senescence-associated beta-galactosidase is lysosomal beta-galactosidase. Aging Cell. 2006;5(2):187–95.
33. Lopez-Otin C, et al. Hallmarks of aging: an expanding universe. Cell. 2023;186(2):243–78.
34. Krtolica A, et al. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. Proc Natl Acad Sci U S A. 2001;98(21):12072–7.
35. Coppe JP, et al. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol. 2008;6(12):2853–68.
36. Baker DJ, et al. Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. Nature. 2016;530(7589):184–9.
37. Chang J, et al. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. Nat Med. 2016;22(1):78–83.
38. Childs BG, et al. Senescent intimal foam cells are deleterious at all stages of atherosclerosis. Science. 2016;354(6311):472–7.