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Zhao Wang *Editor*

Aging and Aging-Related Diseases

Mechanisms and Interventions

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Preface

In humans, aging is ineluctable as well as inexorable. That is really mysterious, because from a biological viewpoint there is no reason why aging should happen. Aging is stranger and more fearful than death, because death is, we could say, for the renewal of the world. Why we could not live healthily to 100 or 150 years old and die suddenly without getting aging or bearing aging-related diseases really puzzle us for centuries and even now.

Maybe someday we all can pass away in our bed with no aging and no diseases. But at any rate, every day you get older. So we must face aging and aging-related diseases now, because they have been becoming severe global health problems with the gradual extension of the average life expectancy of human beings. Although the scientific study of aging started around 100 years ago, we do not have clear answers to some essential questions in this field, for example, what is aging, why we age, and how we age. We have even been debating on whether aging is a disease. Though aging changes are manifested from the molecular to the individual level and from internal to external, no biological biomarkers, diagnostical standards, or therapeutical medications on aging are clearly found or established. But everybody indeed gets older every day with some aging characteristics sooner or later.

We live in a strange world where people are aging and mortal. Though it is not possible to prevent aging, or to reverse aging now, we can intervene in aging, delay the aging process, and reduce the incidence of aging-related diseases. Recently the papers related to aging research increase exponentially, and aging is becoming a hot topic in life science. This book aims to provide with an overview of recent advances in the study of aging and aging-related diseases. It discusses the topics at different levels, from systems to molecules, including cohort, individual, organs, tissues, cells, as well as related proteins and genes. The book also covers the studies on possible biomarkers of aging and antiaging interventions. Of course, our focus is not only to extend biological life span of human beings but also improve the living quality as aging. It is still quite difficult to extend the absolute life span now, but we all are trying our best to make everyone live longer with fewer indispositions. We firmly believe that although death is inevitable, aging can be postponed, attenuated, or even reversed.

I sincerely thank my friend Dr. Peng Zhang, who is a senior editor in Springer Nature, for inviting me to edit this book. Most of my academic friends are the experts or researchers in aging sciences and technologies, so we grouped quickly to

form the team for this book. Here, I would like to express my great gratitude to their hard and efficient work, which made this book perfect and valuable. I would like to thank all members in my laboratory for their supports and cooperation. The book is the fruit of collective wisdom and labor.

I sincerely hope that this book will be helpful for your research, your health, and your life.

Tsinghua Garden, Beijing, China Zhao Wang
2018 Spring

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Systems Biology in Aging Research

1

Xian Xia and Jing-Dong J. Han

Abstract

Systems biology is an approach to collect high-dimensional data and analyze in an integrated manner. As aging is a complicated physiological functional decline in biological system, the methods in systems biology could be utilized in aging studies. Here we reviewed recent advances in systems biology in aging research and divide them into two major parts. One is the data resource, which includes omics data from DNA, RNA, proteins, epigenetic changes, metabolisms, and recently single-cell-level variations. The other is the data analysis methods consisting of network and modeling approaches. With all the data and the tools to analyze them, we could further promote our understanding of the systematic aging.

Keywords

Aging · Systems biology · Network · Omics · Single cell

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1.1 Introduction

Aging is the time-dependent physiological functional decline in all aspects of a biological system, which ultimately leads to death. Systems biology combines computational modeling and simulation, with large-scale experiments, to explore dynamic behavior in biological systems (Cassman 2005), which is an ideal approach to study a systems-level problem like aging. In this chapter we will discuss the data resource and analysis approaches in aging systems biology.

1.2 Data Resource for Systems Biology in Aging Research

The omics data are the basic building blocks for constructing a global view of a tissue or organism in the aging process through systems biology approaches. The following will highlight different omics data source in the aging research and their findings.

1.2.1 Genomics

The genome-wide association studies in aging research are based on genetic variants measured by single nucleotide polymorphism (SNP) chips or high-throughput genome sequencing and phenotypes such as the chronological age- or healthy aging-related phenotypes. A series of twin studies (Paneni et al. 2017; Ljungquist et al. 1998; Skytthe et al. 2003) have shown that 20–30% of the overall variation of human lifespan can be attributed to genetic factors, indicating that lifespan is not genetically determined to a large extent, although the genetic influences on lifespan increase after age 60 (Hjelmborg et al. 2006). With that being said, there are apparently some genetic determinants for longevity. The SNPs on APOE (Gerdes et al. 2000; Ewbank 2007; Deelen et al. 2011; Joshi et al. 2017; Sebastiani et al. 2017) and FOXO3A (Joshi et al. 2017; Willcox et al. 2008; Pawlikowska et al. 2009; Flachsbart et al. 2009; Anselmi et al. 2009) are repeatedly found to be associated with longevity in studies of centenarians versus younger controls. In contrast, a recent GWAS research on healthy aging reveals that healthy aging (in this study defined as people >80 years without chronic diseases and not taking chronic medications) shares no SNP loci with exceptional longevity, suggesting they are very divergent phenomena, although they are intuitively expected to share some common features (Erikson et al. 2016). Instead this study found no major single contributor to healthy aging (Erikson et al. 2016).

1.2.2 Epigenomics

Genome-wide DNA methylation can be measured by chip (Illumnia 450 K or 850 K chip) or sequencing (whole-genome bisulfite sequencing, reduced-representation

bisulfite sequencing, methylated DNA immunoprecipitation sequencing, or methyl-CpG binding domain enriched sequencing) (Bock et al. 2010; Harris et al. 2010). The global pattern of DNA methylation during aging is hypo-methylation in repetitive sequences, hyper-methylation in promoter regions, and higher intercell variability (Bacalini et al. 2014; Cevenini et al. 2008). A study using DNA methylation to estimate the state of aging in blood found that only three CpG sites could predict age with a mean absolute deviation from chronological age of less than 5 years (Weidner et al. 2014), providing a DNA methylation-based aging biomarker. A cross-sectional study that evaluated DNA methylation in boys aged 3–17 years found that >88% pediatric age-associated loci trend in the same direction as in adulthood, suggesting that some of the methylation changes with age take place in early life stages (Alisch et al. 2012). Aging-associated DNA methylation is shared across different tissues within the same individuals, as indicated by one research which found that differentially methylated regions in whole blood can be replicated in buccal cells (Rakyan et al. 2010), and another research found that age-methylation correlations are well preserved between the brain and blood (Horvath et al. 2012).

1.2.3 Transcriptomics

Transcriptome is also measured by either microarrays or RNA sequencing methods. Changes in the aging transcriptome are found to be tissue-specific, as most of the changes from the brain (Lu et al. 2004; Berchtold et al. 2008), skin (Glass et al. 2013), adipose tissue (Glass et al. 2013), kidney (Rodwell et al. 2004), and blood (Peters et al. 2015) did not overlap with other tissues. And the change also shows species specificity, because a cross-species analysis found only 73 genes consistently associated with age (de Magalhães et al. 2009). The repeated biological functions that change in the aging process include increased inflammation and decreased energy metabolism especially mitochondrial functions (Zierer et al. 2015).

1.2.4 Proteomics

Current proteomic techniques based on immunoassays, protein arrays, or mass spectrometry can measure only a small fraction of the proteome (up to 1000 proteins per a sample). The most comprehensive description of the human proteome across various human tissues, cell lines, and body fluids to date consists of 18,097 proteins collected from 16,857 liquid chromatography tandem-mass spectrometry (LCMS/MS) experiments (Wilhelm et al. 2014). Recent research using quantitative middle-down proteomics found that a histone variant H3.3 is accumulated during aging (Tvardovskiy et al. 2017), and another research in *Drosophila* showed that tissue-specific proteome in long-lived mutant strains new insights on the insulin/IGF signaling pathway (Tain et al. 2017). A proteomics study of young and old B cells found that protein related to stress management in mitochondria and DNA repair is under significant regulation during aging (Mayer et al. 2017). Besides the identification of proteins from proteomics data, a distinctive value of such data

source is the posttranslational modification (PTM) information, which cannot be directly measured by any other omics but can alter biochemical properties of proteins. PTM is significantly changed during aging, for example, levels of N-glycosylation correlate with familial longevity and healthy aging (Ruhaak et al. 2011) and linear combination of only three IgG glycans explained up to 58% of variance in age in a research of four European populations (Krištić et al. 2014). As mass spectrometry (MS)-based proteomics fields are more open to data sharing practice, it is the golden age to analyze public proteomics data (Martens and Vizcaíno 2017). OpenMS (Röst et al. 2016) is an open-source tool available to assist such analyses.

1.2.5 Metabolomics

Metabolomics profiles the low-molecular-weight molecules in a biological sample. Similar to proteomics, this profiling is based on either mass spectrometry or nuclear magnetic resonance. To date, there is no analytical method available to determine and quantify all metabolites in a single experiment (Adamski and Suhre 2013). From 2008 till today, a series of metabolomics studies in human aging have been done (Gonzalez-Covarrubias et al. 2013; Lawton et al. 2008; Menni et al. 2013; Yu et al. 2012) in small to large cohorts. A lipidomics study in middle-aged offspring of nonagenarians found that improved antioxidant capacity and more efficient β-oxidation function might be responsible for increased lifespan in women (Gonzalez-Covarrubias et al. 2013), and another study found that C-linked glycosylated tryptophan was highly correlated with age and aging traits, such as lung function, bone mineral density, and blood pressure (Menni et al. 2013). Now metabolomics are often conducted with other layers of omics to facilitate the study, such as in the proteomics study mentioned before, metabolomics are used to verify their conclusions (Mayer et al. 2017).

1.2.6 Metagenomics

The human metagenomics refers to the collective genome of microbial species hosted by the human body. Metagenomics of fecal samples found that the separation of microbiota composition significantly correlated with measures of frailty, markers of inflammation and nutritional status in older people, as well as their residential situation (Claesson et al. 2012).

1.2.7 Phenomics

Phenomics refers to the clinical and lifestyle traits, ranging from anthropometric measures to health and lifestyle questionnaires (Moayyeri et al. 2013). As aging is tightly linked to lifestyle, for example, calorie restriction and exercise are

repeatedly found to slow aging (Green et al. 2017), phenomics is especially valuable in aging research. The Rockwood frailty index, which is composed of symptoms, signs, diseases, and disabilities, could be used as a measure of biological age (Rockwood and Mitnitski 2007). The phenomics could be interdependent on each other, such as faster telomere attrition, and higher inflammaging burden (measured by interleukin-1β) was associated with lower grip strength (Baylis et al. 2014). Recently, the human 3D face was also profiled for the aging study, and features extracted from the 3D such as eye slopes were found to be tightly associated with age, while physical age predicted from the 3D face was found to be more consistent with health indicators than chronological age (Chen et al. 2015).

1.2.8 Single-Cell/Organism Measurement

Although not necessarily through omics approaches, single-cell/organism measurement could also be informative for aging research and suit the need for systems biology as such experiments often generate big dataset for the downstream integrative analysis. The aging-related immune system changes have been investigated via 15-color flow cytometry panel (measures 14 proteins) in 28 T cell subpopulations in human (Lu et al. 2016) and single-cell RNA-seq in naïve and effector memory $CD4^+$ T cells in mice from two divergent species (Martinez-Jimenez et al. 2017). The latter found that aging increases cell-to-cell variation on transcriptome level, which suggests that transcriptomic switch driven by immunological activation is no longer controlled as tight as in young mice (Martinez-Jimenez et al. 2017). Another single-cell RNA-seq study in human pancreas of 2544 single cells from 8 donors spanning six decades of life found that older donors display increased levels of transcriptional noise and potential fate drift (Enge et al. 2017). With the development of micro-fluid technology in model animals such as yeast *S. cerevisiae* (Chen et al. 2017) and worm *C. elegans* (Xian et al. 2013) or other equivalent culture techniques utilizing a polyethylene glycol hydrogel and a silicone elastomer (Pittman et al. 2017), there have been significant efforts to delineate the long-time puzzle about how aging differs among genetically identical individuals within the same species, which reflects the stochastic nature of the aging process.

1.3 Data Analysis for Systems Biology in Aging Research

The data analysis for aging systems biology generally could be separated into two parts: the network approach and the mathematical modeling approach. The following will briefly discuss the advance of the application of such approaches in the aging research and their conclusions.

1.3.1 Network Construction

One way to integrate the result of an omics study in a systems biology context is to project the variables of interest onto known reference networks, such as protein-protein interaction (PPI) networks, gene regulatory networks (GRN), or metabolic networks. PPI can be obtained from the Human Protein Reference Database (Keshava Prasad et al. 2009), the MIPS mammalian protein-protein interaction database (Pagel et al. 2005), the Reactome database (Fabregat et al. 2017), and the STRING database (Szklarczyk et al. 2017). Metabolic networks are mainly from Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al. 2017).

On such predefined network, aging-associated proteins are found to be highly connected hubs in the PPI network (Bell et al. 2009), and type I diabetes is more tightly related to aging than type II diabetes using an asymmetric closeness based on the PPI network (Wang et al. 2009). Through integration of DNA methylation and PPI, tissue-independent age-associated hotspots were found to target stem cell differentiation pathways (West et al. 2013). By restricting the PPI to age-specific highly expressed genes, although the global network topologies did not change, the centrality of several genes correlated with age (Faisal and Milenković 2014). A study from our laboratory analyzed the topology of aging-related PPI subnetwork in which interacting gene pairs are transcriptionally co-expressed or anti-expressed during human brain aging and found that the PPIs connecting anti-expressed genes are enriched for lifespan regulators and transcriptional and epigenetic regulators (Xia et al. 2006).

Another way of network inference is through data-driven approaches, which can be separated into five major classes according to the Dialogue on Reverse Engineering Assessment and Methods (DREAM) project: regression, mutual information, correlation, Bayesian networks, and others (Marbach et al. 2012). One should keep in mind that network inference is at best an indication of association and experimental validations are always needed to demonstrate causality. The following are some examples of network construction efforts in aging research.

The weighted gene co-expression network analysis is a method to infer the gene-gene interaction networks from transcriptomics data (Zhang and Horvath 2005), and by applying the method to gene expression data from 30 adult human frontal cortex samples of different ages and comparing the resulting network to a network derived from AD transcriptome, Miller and colleagues found that healthy aging of the brain and AD share features in the decline or mitochondrial activity and synaptic plasticity (Miller et al. 2008). Such co-expression- or correlation-based network can be also used to integrate multiple layers of data, for example, in a recent effort to profile young and old adults' vaccinal responses, a multiscale, multifactorial response network spanning transcriptomic and metabolomics signatures, cell populations, and cytokine levels was built and reveals striking associations between orthogonal datasets (Li et al. 2017). Similar idea could be generalized to single-cell

transcriptome analysis, as has been done in the SCENIC computational tool, which could simultaneously reconstruct gene regulatory network and identify cellular states (Aibar et al. 2017).

Probabilistic graphical models are an important class of networks that can be built with high-throughput data (Friedman 2004). In a study of metabolomics data, a Gaussian graphical model (GGM) was applied to infer association networks (Krumsiek et al. 2011). GGM is also applied in aging research to reconstruct networks from metabolic data and identify modules (Murphy et al. 2017). A Bayesian network is a directed acyclic graph inferred from data which could extract biological meaningful associations without prior knowledge (Friedman et al. 2000). Recently our laboratory developed an algorithm that could combine the public intervention data to infer a Bayesian network (Li et al. 2013) and applied it to transcriptomic data of *C. elegans* during normal aging and dietary restriction (DR), which led to the finding that there are extensive feedback controls which exist among three modules mediating DR-induced longevity and validated them by lifespan assay (Hou et al. 2016).

1.3.2 Model Aging Systems

The ultimate goal of systems biology is to quantitatively model an organism, conduct in silico experiments, and generate hypotheses and predictions. While whole-organism models have been attempted in yeast (Karr et al. 2012), modeling a subsystem of an organism based on prior knowledge also allows mechanistic insights on the biological process such as aging. A stochastic network model of cell senescence based on telomere reduction, mitochondria damage, and nuclear somatic mutations was built, and the simulation from this model was consistent with published data on intra-clonal variability in cell-doubling potential (Sozou and Kirkwood 2001). The same group also developed a mathematical model to describe the heat shock system and to describe the influence of chaperones and accumulation of misfolded proteins on aging (Proctor et al. 2005). Another modeling work focused on the mitochondrial fission and fusion events and found that the simulation from their model was consistent with two experimental findings so that this model could provide evidences for age-related accumulation of mitochondrial deletion mutants (Kowald et al. 2005). An in silico model of the chronic effects of elevated cortisol on hippocampal atrophy was developed, and simulations using ordinary differential equations suggested that chronic increase in cortisol levels leads to faster decline in hippocampal output than acute bursts (McAuley et al. 2009). The epigenetic changes in aging stem cells were also modeled to explain why increased stem cell proliferation can lead to progeroid phenotypes (Przybilla et al. 2014). One interesting effort besides the researches in the biological side of aging is the facial aging modeling,

which is useful in looking for lost children or wanted fugitives, utilizing four types of approaches: physical model-based approaches, prototyping, function-based approaches, and evaluation targeted approach, and the results were impressive (Suo et al. 2012).

1.4 Conclusions

With the rapid development of various omics mapping methods, and accumulating big data, studying aging at systems biology level is now not only feasible but becoming a necessity to complement traditional one-gene-at-a-time approaches. Aging systems biology (data sources and analysis are summarized in Fig. 1.1) will bring new insights to aging both macroscopically at the network level and microcosmically using mathematical models. Single-cell technology will further fuel the aging systems biology study toward single-cell levels, and linked with big data generated at the cellular, tissue, and whole-organism levels, the time is ripe for aging systems biology to take off and reap fruits.

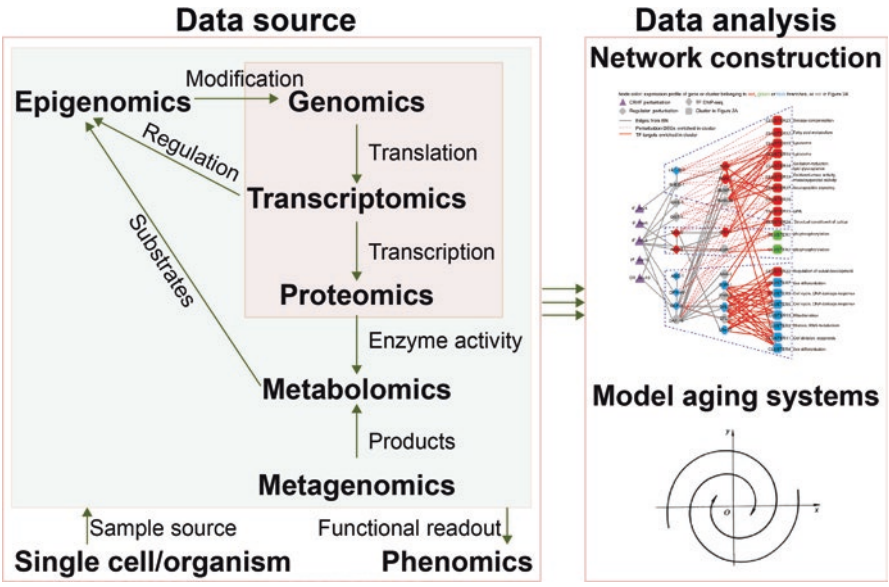

Fig. 1.1 Intervention of data source and analysis in aging systems biology. In this concise sketch map, all the types of data sources and analysis methods are nested in the network to show their interdependency to each other. The network is obtained from Hou et al. (2016)

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Circular RNA in Aging and Age-Related Diseases

2

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Abstract

Circular RNAs (circRNAs) are widely present and participate in a variety of biological regulatory activities as a novel type of endogenous noncoding RNA molecule. With advances in RNA structure and function analysis, it was found that circRNAs are present in a myriad of life processes and longevity in model organisms such as mice, flies, and worms. Accumulating evidence indicates the involvement of circRNAs in regulation of age-related pathologies such as cancer, diabetes, cardiovascular disorders, and neurodegenerative disease, suggesting that circRNAs may have great potential implications in clinical and research fields. In this chapter, we review recent advances in circRNA functions and mechanisms and discuss their roles in aging and age-related diseases. It will provide insight into the regulatory roles of circRNAs in aging and age-related diseases.

Keywords

Circular RNA · Aging · Age-related diseases · Molecular function · Biomarker

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2.1 Introduction

Aging is the primary risk factor for major human pathologies such as cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases (Gems and Partridge 2013; Lipska et al. 2016; De and Ghosh 2017). In the regular aging process and aging-related progression onset, noncoding RNAs play the most important roles (Yang et al. 2016; Elia et al. 2017). Circular RNAs (circRNAs) are a class of noncoding RNAs characterized by the presence of covalently linked ends produced in a noncanonical splicing event called "back-splicing" (Jeck and Sharpless 2014), which possess the pervasive expression from very simple organisms such as fungi to human (Memczak et al. 2013; Wang et al. 2014). Following the technical and methodological advancements in circRNAs research, their functions and mechanisms were confirmed in life processes (Guarnerio et al. 2016). The function of circRNAs is closely related to their structural features. Based on source of circRNAs, they are divided into four categories as shown in Fig. 2.1 A (Qu et al. 2016; Wang et al. 2016; Meng et al. 2017). Current available results demonstrate that circular RNA isoforms are more abundant than linear RNAs from the same locus, raising the intriguing possibilities of functional roles for these molecules (Salzman et al. 2013; Guo et al. 2014). CircRNAs are highly abundant in some tissues and are stable molecules (Memczak et al. 2013; Jeck et al. 2013), indicating versatile functions such as being the regulators of muscle physiology and age-related decline in muscle function (Abdelmohsen et al. 2015).

Increasing evidence supports that circular RNA is involved in biochemical pathways, such as acting as miRNA sponges and binding proteins associated with mRNAs, which modulate their expression posttranscriptionally (Hansen et al. 2013; Ashwal-Fluss et al. 2014). In addition, circRNAs can regulate gene expression at multiple levels (Huang et al. 2017) (Fig. 2.1 B). CircRNAs are also involved in the regulation of physiological aging processes in humans and model organisms (Westholm et al. 2014; Abdelmohsen et al. 2015; Gruner et al. 2016; Panda et al. 2016; Cortés-López et al. 2018). Furthermore, a large body of literature reports that circRNAs are promising potential biomarkers for disease with clinical significance in human cancers due to their unique structure, high stability, and specific expression patterns (Tang et al. 2017a; Fu et al. 2017a, 2018; Wang et al. 2017; Xue et al. 2017; Zhou and Yu 2017).

In this review, we briefly summarized and discussed the function mechanisms of circRNAs in physiological aging processes and age-related diseases.

Fig. 2.1 (continued) contain the translation start site, the remaining truncated linear mRNA will fail to translate and reduce the translation of parental gene indirectly; (5) alternative splicing, the biogenesis of circRNAs can compete against canonical pre-mRNA splicing to facilitate alternative splicing; (6) transcriptional regulation, circRNAs (containing ciRNAs and EIciRNAs) can regulate transcription by directly interacting with RNA polymerase II and U1 snRNP (Li et al. 2015); and (7) m6A-driven translation. A single N6-methyladenosine (m6A) in consensus with RRACH motifs of circRNAs is sufficient to drive translation initiation. (Yang et al. 2017)

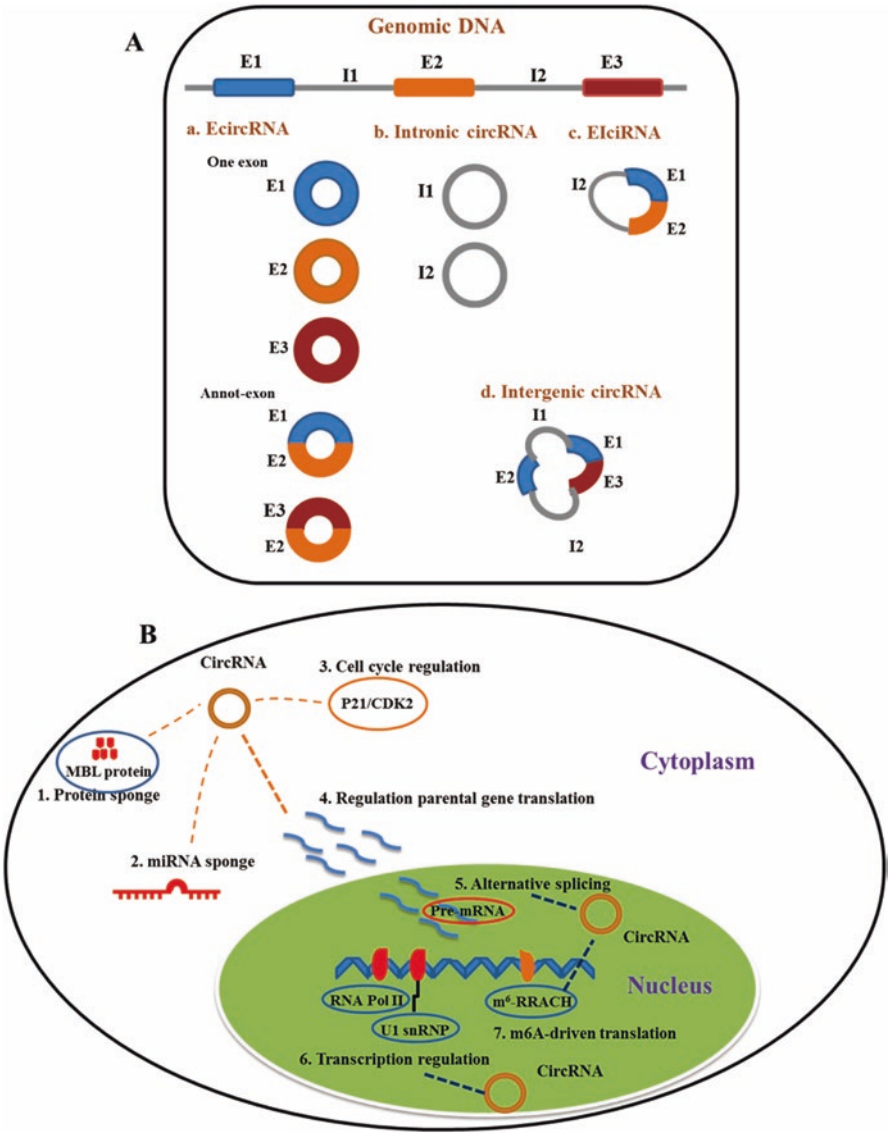

Fig. 2.1 Circular RNA classification and biological functions. (**a**) Based on source of circRNAs, these are divided into four categories: exonic circRNAs (ecircRNA, including one exon and annotated exon), circular RNAs from introns, exon-intron circRNAs (EIciRNA), and intergenic circRNAs. E1, exon 1; E2, exon 2; E3, exon 3; I1, intron 1; I2, intron 2; Annot-exon, annotated exon. (**b**) To date, seven biological functions of circRNAs have been discovered: (1) protein sponge, *circMbl* was reported to bind directly to the muscle blind protein and sponge out the excess MBL protein (Ashwal-Fluss et al. 2014); (2) miRNA sponge, circRNAs acting as a sponge of miRNA can compete with miRNA and reduce its binding to the target genes, thereby regulating the target gene expression indirectly; (3) cell cycle regulation, *circ-Foxo3* can bind to p21 and CDK2 to regulate cell cycle progression (Du et al. 2016); (4) regulation of parental gene translation, if circRNAs

2.2 CircRNAs in the Physiological Aging Processes

Recent studies have indicated that circRNAs are expressed in different tissues, especially in the brain (Westholm et al. 2014; Gruner et al. 2016), muscle tissues (Abdelmohsen et al. 2015), and human fibroblasts and CD8(+)T cells (Wang et al. 2015b; Panda et al. 2016).

2.2.1 CircRNAs Accumulation in the Aged Brain

Recent research results have shown high levels of circular RNA isoforms in the developing human brain (Ng et al. 2013) and the aging fly brain (Westholm et al. 2014). The expression of these circRNAs in humans, mice, and fruit flies has also been validated, showing that preferential circRNA expression in the brain is conserved among species. Also, with the aging process, the accumulation of circRNAs and higher expression level can be observed in those brains (Memczak et al. 2013; Rybak-Wolf et al. 2015; Szabo et al. 2015).

CircRNA induction is specific for the neuronal (not glial cells) and cannot be explained by accumulation in nondividing cells (Rybak-Wolf et al. 2015). *CircRims2*, *circElf2*, and *circDym* were highly expressed in the mouse cerebellum, while *circPlxnd1* was enriched in the cortex (Rybak-Wolf et al. 2015). Subsequently, circRNAs were significantly upregulated in the hippocampus and central nervous system (CNS) in samples of mice (Gruner et al. 2016). Not only does the adult CNS express the highest level of circular RNAs, it continues to accumulate circular RNAs during aging (Calarco et al. 2009; Brown et al. 2014). In *Drosophila*, circRNAs with significantly higher expressions in aging heads were enriched for functional annotations related to neural signaling and developmental process (Westholm et al. 2014). Meanwhile, the *mbl* circle is preferentially expressed in the nervous system and contains highly conserved 5′ UTR sites for several miRNAs, such as neural miR-279 and miR-315. Furthermore, circRNAs are not equally distributed in the neuronal compartments, but are highly enriched in the mouse brain synapses, such as circRNAs from the *ankib1* gene (*mm9_circ_000903*, cortex), *zfp609* gene (*mm9_circ_004501*, cortex), and *circ-Rims2* (synaptoneurosomes) (Rybak-Wolf et al. 2015).

In our unpublished study data, 64 differentially expressed circRNAs between 4 comparative groups were identified, and the majority of circRNAs were specifically expressed in the fly heads (including 7-day dietary restriction (DR) vs 7-day fully fed (FF), 42-day DR vs 42-day FF, 7-day DR vs 42-day DR, 7-day FF vs 42-day FF). These results support that circular RNAs are an aging biomarker in the CNS. Their specific expression of circRNAs and their stability make them very interesting candidates as biomarkers for neurodegenerative diseases such as Alzheimer's disease (Lukiw 2013).

2.2.2 CircRNAs Abundant in Skeletal Muscle

Skeletal muscle undergoes dramatic changes in the aging process, such as loss of muscle mass, reduced strength, and impaired ability to regenerate (Karakelides and Nair 2005; Tosato et al. 2007). Two hundred and eighteen circRNAs were identified from 24 primate muscle samples spanning a range of ages (including young, middle-aged, and old age). These circRNAs were further studied to assess possible changes in circRNA abundance with age. The results suggested that the abundance of the majority of muscle circRNAs does not change with increasing age, but a specific subset of circRNAs did appear to show declining levels with age (including *mmu_circ_017332*, *mmu_circ_014269*, *mmu_circ_015060*, *mmu_circ_006895*, and *mmu_circ_014509*) (Abdelmohsen et al. 2015). These results contribute to the exciting emergence of circRNAs as versatile regulators of gene expression in biological and clinical frameworks, including muscle physiology and the age-related decline in muscle function.

2.2.3 CircRNAs in Fibroblasts and CD8(+)T Cell

The accumulation of senescent cells has been associated with disease processes such as sarcopenia, arthritis, cancer, diabetes, and neurodegeneration (Tchkonia et al. 2013; Baker et al. 2016). *CircPVT1* demonstrated markedly reduced levels in senescent (late-passage) human diploid WI-38 fibroblasts. Reduced *circPVT1* levels in proliferating fibroblasts triggered senescence, as suggested by a rise in senescence-associated β-galactosidase activity, higher abundance of CDKN1A/P21 and TP53, and reduced cell proliferation (Panda et al. 2016). Meanwhile, the senescence-associated circRNA *circPVT1* binds to *let-7* and modulates *let-7*-regulated mRNAs (*IGF2BP1*, *KRAS*, and *HMGA2*), thereby influencing senescence (Panda et al. 2016).

An increase in the CD28(−)CD8(+) T subset is considered to be one hallmark of immunosenescence (Parish et al. 2010). CircRNA profiles in CD28-dependent CD8(+)T cell subsets of elderly individuals (90.5 ± 2.4–69.3 ± 3.1 year old) and adult controls (32.0 ± 4.6 year old) were identified (Wang et al. 2015b). In this study, *circRNA100783* may be involved in the loss of CD28 in CD8(+)T cells during aging and may regulate phosphoprotein-related signal transduction in CD28-dependent CD8(+) T cell aging, as a potential intracellular biomarker of immunosenescence (Wang et al. 2015b).

2.2.4 CircRNAs in *Caenorhabditis elegans*

1112 canonical circular RNAs of *Caenorhabditis elegans* from different development stages (embryo, larval 1~larval 4, and adult stages) demonstrated developmental specificity and had no obvious correlation with linear RNA (Liu and Chen 2015). Of the total 1112 canonical circRNAs, only 119 circRNAs were expressed in

all developmental stages. Therefore, these circRNAs may play a role in development and aging of *Caenorhabditis elegans.*

2.3 Cancer-Related CircRNAs

CircRNAs are differentially expressed in a wide range of cancers and could be used as new biomarkers for diagnosis and treatment targets for cancers (Table 2.1).

2.3.1 Hepatocellular Carcinoma

Most circRNAs were downregulated in hepatocellular carcinoma (HCC) tissues, such as *hsa_circ_0005986* (Fu et al. 2017a), *hsa_circ_0004018* (Fu et al. 2017b), *circZKSCAN1* (Yao et al. 2017a), *hsa_circ_0001649* (Qin et al. 2016), and *ciRS-7* (also termed as *Cdr1as*) (Xu et al. 2017). On the other hand, *hsa_circ_001569* (Jin et al. 2017a) and *hsa_circ_0005075* (Shang et al. 2016) expression levels were significantly higher in HCC tissues. The low expression levels of *hsa_circ_0005986*, *hsa_circ_0004018*, *hsa_circ_0005075*, and *hsa_circ_0001649* were correlated with tumor diameters, microvascular invasion, and Barcelona Clinic Liver Cancer (BCLC) stage (Shang et al. 2016; Qin et al. 2016; Fu et al. 2017a, 2018). The *ciRS-7* was significantly correlated with the following three clinicopathological characteristics of HCC patients and hepatic microvascular invasion (MVI) (Xu et al. 2017). Low expression level of *ZKSCAN1* was only associated with tumor size. Additionally, the *circZKSCAN1* levels varied in patients with different number of tumors, liver cirrhosis, vascular invasion, and tumor grade (Yao et al. 2017a).

CircRNAs have multiple action mechanisms in HCC because the miRNA sponge affects host gene expression. For example, *hsa_circ_0005986* downregulation liberated miR-129-5p and decreased the expression level of *Notch1* mRNA (Fu et al. 2017a). Therefore, *hsa_circ_0004018*-miR-30e-5p/miR-626-MYC axis may play a role in HCC carcinogenesis and metastasis (Fu et al. 2017a). Furthermore, these circRNAs are not only used as novel biomarkers for diagnosis and potential therapeutic targeting but also may play important roles in HCC. The *circZKSCAN1* and *hsa_circ_0001649* expressions could serve as the biomarkers for distinguishing cancerous tissue from adjacent non-cancerous liver tissue (Qin et al. 2016; Yao et al. 2017a). Moreover, *hsa_circ_0005075* showed good sensitivity and specificity (Shang et al. 2016), proving to be a potential diagnostic target for HCC.

2.3.2 Gastric Cancer

Specific expression levels and function mechanisms of several circRNAs in gastric cancer (GC) have been identified. *Hsa_circ_0001649* (Li et al. 2017c), *hsa_circ_0001895* (Shao et al. 2017a), *hsa_circ_0000705* (Shao et al. 2017b), *hsa_circ_000019* (Chen et al. 2017c), and *hsa_circ_0000096* (Li et al. 2017a) were

Table 2.1 CircRNAs in human cancers

Human cancer	CircRNAs	Comments	Refs.
Hepatocellular carcinoma (HCC)			
	Hsa_circ_0003570	Low expression in HCC cell lines and HCC tissues	Fu et al. (2018)
	Hsa_circ_0005986	Low expression accelerates cell proliferation by promoting the G0/G1 to S phase transition as a miR-129-5p sponge	Fu et al. (2017a)
	Hsa_circ_0004018	Low level triggers carcinogenesis and metastasis by *hsa_circ_0004018*-miR-30e-5p/miR-626-MYC axes	Fu et al. (2017b)
	Hsa_circ_001569	High expression accelerates the proliferation of HCC cells	Jin et al. (2017a)
	Hsa_circZKSCAN1	Overexpression of *circZKSCAN1* effectively inhibits HCC cell proliferation, invasion, and migration	Yao et al. (2017a)
	Hsa_circ_0005075	Higher levels in HCC tissues act as miR-23b-5p, miR-93-3p, miR-581, miR-23a-5p sponge	Shang et al. (2016)
	Hsa_circ_0001649	Low expression positively correlates with the HCC metastasis	Qin et al. (2016)
	CiRS-7(Cdr1as)	Low *ciRS-7* in HCC tissue act as a miR-7 sponge and have two targets (PIK3CD and p70S6K)	Xu et al. (2017)
Gastric cancer (GC)			
	Hsa_circ_0001649	Low level positively correlates with the GC metastasis	Qin et al. (2016)
	CircPVT1	High level promotes cell proliferation by acting as a miR-125 family sponge and inhibiting its activity	Chen et al. (2017a)
	Hsa_circ_0001895	Low expression in GC cell lines	Shao et al. (2017a)
	Hsa_circ_0000705	Low expression in GC and dysplasia tissues	Shao et al. (2017b)
	Hsa_circ_0000190	Low level in GC tissues and plasma samples from GC patients	Chen et al. (2017c)
	Hsa_circ_0000096	Affects GC cell growth and migration by regulating cyclin D1, CDK6, MMP-2, and MMP-9	Li et al. (2017a)
Laryngeal cancer			
	Hsa_circ_100855	High level in laryngeal squamous cell cancer tissues	Xuan et al. (2016)
	Hsa_circ_104912	Low expression in laryngeal squamous cell cancer tissues	Xuan et al. (2016)

(continued)

Table 2.1 (continued)

Human cancer	CircRNAs	Comments	Refs.
Colorectal cancer (CRC)			
	Hsa_circ_001569	High expression inhibits miR-145 and upregulates its targets E2F5, BAG4, and FMNL2 proteins to promote the proliferation and invasion of CRC cells	Xie et al. (2016)
	Hsa_circ_0000069	Low level inhibits cell proliferation, migration, and invasion and induces G0/G1 phase arrest of cell cycle	Guo et al. (2016)
	Hsa_circ_001988	Low expression level in CRC tissues	Wang et al. (2015a)
	CDR1as	Low *CDR1as* suppresses CRC cell proliferation and invasion by acting as a miR-7 sponge and positively regulating EGFR and IGF-1R	Tang et al. (2017b)
Clear cell renal cell carcinoma (ccRCC)	*CircHIAT1*	Low *circHIAT1* in ccRCC and AR-*circHIAT1*-miR-195-5p/29a-3p/29c-3p signals function through CDC42 expression to regulate ccRCC cell migration and invasion	Wang et al. (2017)
Oral squamous cell carcinomas (OSCC)	*CircRNA_100290*	Low expression inhibits proliferation of OSCC cell lines in vitro and in vivo by directly binding to miR-29 family member	Chen et al. (2017b)
Bladder carcinoma	*CircTCF25*	Promotes the proliferation, migration, tumor growth, and metastasis by *circTCF25*-miR-103a-3p/miR-107-CDK6 pathway	Zhong et al. (2016)
Esophageal squamous cell carcinoma (ESCC)	*Hsa_circ_0067934*	High *hsa_circ_0067934* promotes the motility and migration of ESCC cells and affects cell cycle status	Xia et al. (2016)
Acute myeloid leukemia(AML)	*Hsa_circ_0004277*	Lower expression level of *hsa_circ_0004277* in AML	Li et al. (2017b)
Osteosarcoma	*Hsa_circ_0016347*	High expression level promotes the proliferation, invasion, and metastasis of osteosarcoma cells as a natural miR-214 sponge to indirectly influence the caspase-1 expression	Jin et al. (2017b)
Lung cancer	*Hsa_circ_100876*	High level in non-small cell lung cancer tissues	Yao et al. (2017b)
Breast cancer	*Hsa_circ_Foxo3*	High expression level suppresses cancer cell proliferation, survival, and progression of breast cancer cells	Lu (2017)
Cervical carcinoma	*CircPABPN1*	High levels suppresses HuR binding to *PABPN1* mRNA	Abdelmohsen et al. (2017)

significantly downregulated in GC tissue, serum samples, or cell lines. *Hsa_circ_0001895* (Shao et al. 2017a) level was related to cell differentiation, Borrmann type, and tissue carcinoembryonic antigen (CEA) expression, but not associated with other important clinicopathological features, such as tumor diameter, invasion, or TNM stage. *Hsa_circ_0000705* (Shao et al. 2017b) and *hsa_circ_0000190* (Chen et al. 2017c) expression levels were also strongly associated with tumor location, tumor stage, Borrmann type, pathologic diagnosis, and tissue CA19-9 expression.

Out of the circRNAs discussed above, the specific action mechanism of *circPVT1* and *hsa_circ_0000096* was clearly understood (Chen et al. 2017a; Li et al. 2017a). For instance, knockdown of *hsa_circ_0000096* significantly repressed GC cell proliferation and migration and reduced the protein levels of *cyclin D1*, *cyclin-dependent kinase 6* (*CDK6*), *matrix metalloproteinase-2*, and *MMP-9* in vitro and in vivo (Li et al. 2017a). The circRNAs expressions indicate that these could be used as the biomarker of GC. *Hsa_circ_0001649* and *hsa_circ_0000190* (Chen et al. 2017c) could be used as the essential diagnostic targets. Furthermore, *hsa_circ_0000705* plays a crucial role in gastric carcinogenesis and could be used as an indicator of gastric cancer (Shao et al. 2017b).

2.3.3 Colorectal Cancer

A total of four circRNAs, including three upregulated (*circ_001569*, *hsa_circ_0000069*, and *CDR1as*) and one downregulated (*hsa_circ_001988*), were found in colorectal cancer (CRC) tissues. Higher expression levels of *circ_001569* (Xie et al. 2016), *hsa_circ_0000069* (Guo et al. 2016), and *CDR1as* (Tang et al. 2017b) were closely correlated with CRC pathology traits. For example, differentiation and TNM classification increased along with the progression of T classifications, N classifications, distant metastasis, etc. Conversely, the lower expression of *hsa_circ_001988* was only related to differentiation and perineural invasion (Wang et al. 2015a). These circRNAs could be exploited as the biomarkers in the progression of CRC, as promising targets involved in diagnosis and therapy.

2.3.4 Other Cancers

The specific circRNAs were identified successively in esophageal squamous cell carcinoma (ESCC), non-small cell lung cancer (NSCLC), acute myeloid leukemia (AML), breast cancer, and cervical carcinoma. These could represent the potential biomarkers and therapeutic targets of those cancers as several important circRNAs were found.

In oral squamous cell carcinomas (OSCC), *circRNA_100290* may function as a competing endogenous RNA to regulate *CDK6* expression by sponging the miR-29b family members (Chen et al. 2017b). In bladder carcinoma, *circTCF25* can play a positive regulatory role on *CDK6* to stimulate cell proliferation in bladder cancer

(Zhong et al. 2016). In AML and breast cancer, *hsa_circ_0004277* (Li et al. 2017b) and *hsa_circ_Foxo3* (Lu 2017) were upregulated, respectively. *Hsa_circ_0004277* could also act as a miRNA sponge and regulate its own circRNA-miRNA-mRNA network associated with successful treatment, offering a potential treatment target in AML (Li et al. 2017b). Furthermore, *hsa_circ_Foxo3* can upregulate Foxo3 protein levels by two different mechanisms, regulates cellular functions of heart tissue cells, and suppresses cancer cell progression (Lu 2017).

In cervical carcinoma, *circPABPN1* has been cited as the first example of competition between a circRNA and its cognate mRNA for RNA-binding proteins that affect translation (Abdelmohsen et al. 2017). *CircPABPN1* as the most prominent HuR target circRNA arises from the *PABPN1* pre-mRNA. Moreover, *circRNA_100284* via miR-217 mediated regulation of EZH2, which is involved in the arsenite-accelerated cell cycle of human keratinocytes in carcinogenesis (Xue et al. 2017).

2.4 Cardiovascular Disease-Related CircRNAs

CircRNAs are involved in aging-related cardiovascular disease, so it would be an essential target for treatment and diagnosis of the disease.

2.4.1 CircRNA in Cardio-Related Disease

Three circRNAs have been documented to take part in regulation of cardio-related disease, such as cardiac hypertrophy, myocardial fibrosis, and myocardial infarction (MI). Heart-related circRNA (*HRCR*) acts as an endogenous miR-223 sponge to inhibit cardiac hypertrophy and heart failure. It acts as a positive regulator of cardiac hypertrophy, which results in the increase of ARC expression – a target for miR-223 (Wang et al. 2016). *CircRNA_010567* is involved in the pathological fiber formation process, and *circRNA_010567*/miR-141/TGF-b1 axis plays an important regulatory role in the diabetic mice myocardial fibrosis (Zhou and Yu 2017). A *mmu_circ_008396* circRNA orthologue in mice and humans was significantly downregulated in post-myocardial infarction (MI) mice model and ischemic cardiomyopathy patients, respectively (Garikipati et al. 2016). Overexpression of *mmu_circ_008396* significantly enhanced tube formation and reduced apoptosis of human umbilical vein endothelial cells. These results suggested that *HRCR* and *mmu_circ_008396* circRNA might be novel potential targets to prevent cardiac remodeling and also highlight the significance of circRNAs in cardiovascular diseases.

There were another two important circRNAs in cardio-related diseases. C*dr1as* and miR-7a were both upregulated in MI mice with increased cardiac infarct size or cardiomyocytes under hypoxic treatment (Geng et al. 2016). There is another circRNA (*circ-Foxo3*) highly expressed in heart samples of aged patients (Du et al. 2016), which may be targeted for drug development for the inhibition of tissue senescence.

2.4.2 CircRNA in Vascular Disease

Several circRNAs were identified in vascular disease. Circular antisense noncoding RNA in the INK4 locus (*circANRIL*) may act as protective factor against human atherosclerosis (Holdt et al. 2016). *CircANRIL* controlled atheroprotective cell functions through several molecular mechanisms, independent of cis-regulation at *9p21* and miRNA sponging. This is a potential therapeutic target for the treatment of atherosclerosis to promote anti-atherogenic cell functions and is particularly stable against degradation. *Hsa-circ-000595*, *hsa_circ_0124644*, and *circRNA-16* were upregulated in aortic aneurysms (Zheng et al. 2015), carotid plaque ruptures (Zhao et al. 2017a), and the peripheral blood of coronary artery disease (CAD) patients (Bazan et al. 2014) compared to that in normal tissues, respectively. Knockdown of *hsa-circ-000595* (as the miR-19a sponge) may potentially reduce the occurrence of aortic aneurysms (Zheng et al. 2015). In carotid plaque rupture pathology, *circRNA-16*/miR-221 axis may be important in fibrous cap degradation and rupture during the transition from a stable to an unstable carotid atherosclerotic plaque (Bazan et al. 2014). Finally, *hsa_circ_0124644* had the diagnostic value as candidate biomarkers of CAD (Zhao et al. 2017a). Compared with *hsa_circ_0124644* alone, the combination of *hsa_circ_0124644* and *hsa_circ_0098964* as a biomarker had a higher diagnostic value for CAD, making *hsa_circ_0124644* a powerful tool in the diagnosis of CAD.

There were two circRNAs related to vascular endothelial cell proliferation and apoptosis. *Hsa_circ_0010729* regulates vascular endothelial cell proliferation and apoptosis by targeting the miR-186/HIF-1a axis (Dang et al. 2017). Vascular endothelial dysfunction circRNA (*cZNF609*) decreased retinal vessel loss and suppressed pathological angiogenesis in vivo, while increasing the endothelial cell migration and tube formation, along with protecting endothelial cells against oxidative stress and hypoxia stress in vitro (Liu et al. 2017). Moreover, aberrant regulation of *cZNF609* expression was observed in the clinical samples of patients with hypertension and coronary artery disease. Therefore, intervention of *cZNF609* expression could be a promising therapy for vascular dysfunction.

2.5 Diabetes-Related CircRNAs

Aging is found to be associated with the increased occurrence of diabetes (Bloomgarden and Ning 2013). CircRNA expression profile of 14 patients with type 2 diabetes mellitus (T2DM) has recently been identified (Jiang et al. 2017). CircRNAs could help us to understand the pathogenesis of T2DM and could represent novel molecular targets for clinical diagnosis and therapy. To date, several circRNAs have been found to participate in regulation of diabetes.

2.5.1 *CircRNA_000203*

Tang et al. reported that *circRNA_000203* was upregulated in the diabetic mouse myocardium and in Ang-II-induced mouse cardiac fibroblasts (CFs). *CircRNA_000203* could specifically increase the expression of fibrosis-associated genes (*Col1a2*, *Col3a1*) and *α-SMA* in CFs through abolishing the interaction of miR-26b-5p with the targets of *Col1a2* and *CTGF*. Additionally, the expression of *Col1a2*, *Col3a1*, *α-SMA*, and *CTGF* was significantly suppressed by miR-26b in mouse CFs. *CircRNA_000203* might be a potential target for prevention and treatment of cardiac fibrosis in diabetic cardiomyopathy (Tang et al. 2017a).

2.5.2 *Cdr1as*

The *cdr1as* expression has direct inhibition on miR-7 activity in islet cells. Overexpression of *ciRS-7* increased the insulin content compared to the control of mouse islet cells, while the miR-7 expression resulted decrease of insulin content, which demonstrated that *cdr1as* is a specific repressor of miR-7 in the insulin pathway. In contrast, *cdr1as* overexpression was found to increase insulin secretion in freshly isolated mouse islets. The effect of *cdr1as* on insulin content is through insulin biosynthesis, in which potential target genes (*myrip* and *pax6*) of miR-7 may actually play an important role (Xu et al. 2015). *Cdr1as* may represent a useful tool in addressing the growing need of new therapeutic strategies based upon insulin secretion and β cells renewal in diabetes.

2.5.3 *Hsa_circ_0054633*

Zhao et al. reported that 489 circRNAs differentially expressed between healthy individual and T2DM groups. They further characterized *hsa_circ_0054633* and found that it was upregulated in the peripheral blood of prediabetes and T2DM patients by qPCR. Therefore, this circRNA could be taken as a potential diagnostic biomarker for prediabetes and T2DM in clinical practice (Zhao et al. 2017b).

2.5.4 *Hsa-circRNA11783-2*

Hsa-circRNA11783-2 is closely related to both CAD and T2DM (Li et al. 2017d). MiR-608 and miR-3907 have some binding sites for *hsa-circRNA11783-2*, which are associated with tumors and the female reproductive tract (Creighton et al. 2010; Cummins et al. 2006). Furthermore, the precise roles of *hsa-circRNA11783-2* in T2DM require further exploration in future studies.

2.5.5 *CircHIPK3*

CircHIPK3 expression was significantly upregulated in diabetic retinas and retinal endothelial cells following stress related to diabetes. Retinal endothelial cell viability, proliferation, migration, and tube formation in vitro were changed by silencing or overexpressing *circHIPK3*. *CircHIPK3* acted as an endogenous miR-30a-3p sponge to sequester and inhibit miR-30a-3p activity, which led to increased endothelial proliferation and vascular dysfunction (Shan et al. 2017). These data suggest that *circHIPK3* could be a potential target to control diabetic proliferative retinopathy.

2.6 Neurodegenerative Disease-Related CircRNAs

Previous studies have shown that circRNAs accumulate in aged brain tissue such as synapses (Memczak et al. 2013; Rybak-Wolf et al. 2015), which hint toward possible role of circRNAs in neurodegenerative disease pathologies.

2.6.1 Glioma Malignancy

Circ-TTBK2 was positively correlated with the pathological grades of glioma. Overexpression of *circ-TTBK2* promoted cell proliferation, migration, and invasion while inhibiting apoptosis. C*irc-TTBK2* acted as miR-217 sponge in a sequence-specific manner. In addition, upregulated *circ-TTBK2* decreased miR-217 expression, and there was a reciprocal negative feedback between them in an Argonaute2-dependent manner. Besides, reintroduction of miR-217 significantly reversed *circ-TTBK2*-mediated promotion of glioma progression. *HNF1β* was a direct target of miR-217 and played an oncogenic role in glioma cells. Particularly, *circ-TTBK2* knockdown combined with miR-217 overexpression led to tumor regression in vivo (Zheng et al. 2017).

2.6.2 Alzheimer's Disease

CircRNA as the important noncoding RNA may play a role in Alzheimer's disease (AD) (Lukiw 2013). MiRNA-7 is highly abundant in human brain and associated with a circRNA (*ciRS-7*) for miRNA-7 in the same tissues (Lukiw et al. 2012; Hansen et al. 2013). Deficits in *ciRS-7* "sponging activities" might be expected to increase ambient miRNA-7 levels in AD-affected brain cells to ultimately contribute to the downregulation of selective miRNA-7-sensitive mRNA targets (Cogswell et al. 2008). The presence of upregulated miRNA-7, due to a deficiency in *ciRS-7* "sponging" effects, has a high probability to downregulate AD-relevant targets, the

ubiquitin protein ligase A (*UBE2A*) (Bingol and Sheng 2011; Lonskaya et al. 2013). Deficits in other circRNA-mediated "miRNA sponging systems" and ambient upregulation of specific inducible miRNAs may help explain the widely observed, generalized, and progressive downregulation of gene expression that is characteristic of the sporadic AD (Loring et al. 2001; Colangelo et al. 2002; Ginsberg et al. 2012).

2.7 Conclusions

At the present time, effective therapeutics and healthcare issues associated with aging and age-associated diseases have become one of the major concerns in research. CircRNAs have been shown to be excellent therapeutic targets and diagnostic biomarkers of in many diseases. It is known that circRNAs are highly enriched in the biological processes in organisms and continue to accumulate in different tissues and cells during aging. Strong evidence shows that circRNAs play important roles in the modulation of the aging and age-related diseases. The specific circRNAs related to aging have been identified, which have highlighted their important roles in the aging process.

However, the biological functions and mechanisms of most circRNAs largely remain unexplored. Thus, future research should focus on these aspects particularly in aged brain tissue. More circRNAs in age-related diseases are increasingly being identified as the treatment targets and diagnostic biomarkers of pathologies, although the therapeutic application of these circRNAs remains in the early research phase. The maximum therapeutic potential of circRNAs for age-related diseases would be in clinical treatment and diagnosis of patients, which requires deeper clinical studies in this field. CircRNAs related to diabetes and neurodegenerative diseases have fewer reports than other age-related diseases, which suggests that more ground work is needed for these disorders. These efforts are necessary to deepen understanding of the biology of aging and their mechanisms.

New functions for circRNAs are emerging, such as protein sequestration, transcriptional regulation, and potential functions in cancer. However, the function mechanism of most circRNAs to act as sponges of related miRNAs has not been widely exploited. Other new mechanisms, including binding protein and affecting the host gene expression, need to be carried out urgently to discover additional action mechanisms. Further study on multiple circRNA function mechanisms will improve our understanding about aging and age-related diseases.

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Noncoding RNAs in Cardiovascular Aging

3

Yongqin Li, Yujiao Zhu, Guoping Li, and Junjie Xiao

Abstract

With a progressively growing elderly population, aging-associated cardiovascular diseases and other pathologies have brought great burden to the economy, society, and individuals. Therefore, identifying therapeutic targets and developing effective strategies to prevent from cardiovascular aging are highly needed. Accumulating evidences suggest that noncoding RNAs (ncRNAs) such as microRNAs and long noncoding RNAs (lncRNAs) play important roles in regulating gene expression, which contributes to many pathophysiological processes of cellular senescence, aging, and aging-related diseases in cardiovascular systems. Here we provided a general overview of ncRNAs as well as the underlying mechanisms involved in cardiovascular aging. Although the importance of ncRNAs in cardiovascular aging has been reported and commonly acknowledged, further studies are still necessary to elucidate the underlying molecular mechanisms.

Keywords

Noncoding RNAs · Cardiovascular aging · MicroRNAs · LncRNAs · Senescence

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3.1 Introduction

The elderly population (aged over 65) is experiencing a dramatic increase around the world (North and Sinclair 2012). Cardiovascular diseases are the leading cause of death worldwide, and the mortality and morbidity of cardiovascular diseases are significantly increased in elderly population (Laslett et al. 2012). The physiological changes of cardiovascular aging mainly include left ventricular hypertrophy, diastolic dysfunction, increased cardiac fibrosis, prevalence of atrial fibrillation and arterial stiffening, as well as decrease of arterial compliance and maximal exercise capacity (Dai et al. 2012; Laina et al. 2017). Cardiovascular aging makes the heart more sensitive to stress such as diabetes, smoking, hypertension, hypercholesterolemia, and other cardiovascular risk factors (Dai et al. 2012). Better understanding of cardiovascular aging will help delay the progression and reduce the adverse outcome of cardiovascular diseases.

Aging is a multifactorial process, and the study of noncoding RNAs (ncRNAs) has provided novel molecular insights into cardiovascular aging. ncRNAs, according to their length, are classified as short ncRNAs (19–31 nucleotides), midsize ncRNAs (~20–200 nucleotides), and long noncoding RNAs (more than 200 nucleotides) (Esteller 2011). To be specific, ncRNAs are comprised of microRNAs (miRNAs, miRs), PIWI-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), tRNA derivatives, long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs). Among these ncRNAs, miRNAs and lncRNAs have been most extensively studied in different contexts, including aging-associated physiologic processes and disease conditions (Gupta et al. 2014). ncRNAs regulate gene expression through a variety of mechanisms at transcriptional and/or posttranscriptional levels (Krol et al. 2010; Yoon et al. 2013; Salmanidis et al. 2014). Considering aberrant gene expression underlies cardiovascular aging, it is critically important to understand how gene expression is modulated by ncRNAs in diverse aging-associated processes.

In this review, we will summarize the current knowledge about the roles and regulatory mechanism of ncRNAs (miRNAs and lncRNAs) in cardiovascular aging.

3.2 Cardiac Aging

According to the study of apparently healthy individuals, aging results in an increase in left ventricular (LV) wall thickness and a decline in diastolic function (Chiao and Rabinovitch 2015). These changes are regarded as intrinsic cardiac aging, which is independent of conventional risk factors for cardiovascular diseases such as smoking, hypertension, blood lipid levels, and diabetes. To maintain the LV filling reduced by the increase of LV wall thickness, atrial contraction and atrial pressure are gradually increased in aging, which adversely promotes atrial hypertrophy and an increase prevalence of atrial fibrillation (Lakatta and Levy 2003a, b; Lakatta 2003). Impaired early diastolic filling and an increased atrial contraction will gradually induce diastolic dysfunction, which is highly prevalent in older populations

(Bursi et al. 2006; Swinne et al. 1992). Diastolic dysfunction also makes older adults be susceptible to diastolic heart failure, which has been defined as heart failure with preserved ejection fraction (HFpEF) (Kitzman 2002; Brouwers et al. 2012). Although heart rate and systolic function measured by the ejection fraction remains steady in older populations at rest, they are declined during exhaustive exercise (Fleg et al. 1995). This indicated the reduced cardiac reserve in older adults.

3.3 Vascular Aging

In addition to cardiac aging, vascular aging is also an important risk factor for cardiovascular diseases and impacts the process, mortality, and severity of various cardiovascular diseases. Increasing age is associated with arterial stiffness, endothelial dysfunction, increased fibrosis, impaired angiogenesis, and arterial remodeling (Magenta et al. 2013; Schraml and Grillari 2012; AlGhatrif and Lakatta 2015). Thus, hypertension and atherosclerosis are extremely prevalent in the elderly adults, which substantially contribute to functional and structural changes of the vascular system (Marigliano et al. 1993).

During aging, progressive loss of elastin and increase of collagen fibers make the wall of large arteries thicker and less elastic (Sun 2015; Camici et al. 2015). Endothelial cell (EC) and vascular smooth muscle cell (VSMC) dysfunction and apoptosis also contribute to the biomechanical and structural alterations of the vascular wall, which is tightly linked to vascular aging (Menghini et al. 2014). Endothelial cells, the inner layer of blood vessels, regulate multiple aspects of vessel function and serve as a barrier between vessel and the blood stream (Eckers and Haendeler 2015). Endothelial dysfunction, such as senescence, apoptosis, proliferation, and inflammation, reduces the antithrombotic and vasodilatory properties of vascular system during aging (Rippe et al. 2012; Magenta et al. 2013). By contracting or relaxing, VSMCs control blood flow according to external stimuli. With increasing age, VSMCs change from the contractile and quiescent phenotype to a synthetic phenotype, which is characterized by migration into the intima, subsequent proliferation, and extracellular matrix synthesis in the elderly (Monk and George 2015).

3.4 MicroRNAs in Cardiac Aging

Increasing evidences from the last decades have gained deep insights about the roles of miRNAs in various physiological and pathological processes including cardiovascular aging. miRNAs are short (~22 nt) single-stranded ribonucleic acids, mediating translational repression and mRNA degradation mainly by forming partial base pairing with mRNAs (Carthew and Sontheimer 2009; Chekulaeva and Filipowicz 2009). miRNAs are transcribed by RNA polymerase II or III, catalyzed by Drosha/DGCR8 complex and nucleocytoplasmic exported by exportin-5. Following second cleavage by Dicer, miRNAs are processed into ~22 nt RNA

duplex in cytoplasm. Then the duplex loaded onto an Argonaute protein and complemented to the target mRNA, subsequently forming the miRNA-induced silencing complex (miRISC) (Yates et al. 2013). These miRNA machinery proteins have been shown important for cardiac aging. Mice lacking DGCR8 in muscle tissue demonstrated the premature signs of heart failure and dilated cardiomyopathy, which are usually prevalent in elderly (Rao et al. 2009). Argonaute proteins (AGO1 and AGO2) have been shown to increase with age in mouse hearts (Zhang et al. 2012). Therefore, the upstream regulation of miRNAs might play important roles in cardiac aging.

In human studies, the group of Daniel Levy performed whole-blood miRNA profiling in 5221 adults and identified 127 miRNAs that were differentially expressed with increase of age, which have potential utility to detect accelerated aging and to predict risks for age-related diseases (Huan et al. 2016). In mouse hearts, the group of Thomas Thum performed miRNA array analysis in male healthy mice at neonatal, 4 weeks, 6 months, and 19 months old. miR-22 and miR-24 were significantly increased, while miR-351 and miR-542 were decreased in postnatal hearts compared with neonatal controls (Jazbutyte et al. 2013). Another study compared aged mice (18–20 months old) with young mice (6–8 weeks old) and identified a number of dysregulated miRNAs such as miR-21, miR-34a, miR-574, miR-146a, and miR-29 (Boon et al. 2013). miRNA array analysis of healthy young mice (4-month-old) and old mice (24-month-old) also found that 65 miRNAs were differentially expressed in ventricular tissues (Zhang et al. 2012). These results imply that the dynamic changes of miRNAs were present in heart tissues during aging.

Up to now, the significance and mechanisms of several miRNAs in cardiac aging have been elucidated (Table 3.1 and Fig. 3.1). At the cellular level, aging alters the functional characteristics of cardiomyocytes and cardiac fibroblasts, and these cellular changes contribute to age-dependent alterations of the heart (Lee et al. 2015). miR-22 was prominently increased in aging mouse hearts, and overexpression of miR-22 induced cellular senescence and promoted migratory activity without affecting cell proliferation and apoptosis in neonatal rat cardiac fibroblasts (Jazbutyte et al. 2013). Mimecan (osteoglycin, OGN) was validated as a bona fide target of miR-22 and partially mediated the miR-22 effects in cardiac fibroblasts (Jazbutyte et al. 2013). In addition, miR-22 was also enriched in smooth muscle cells, and miR-22 inhibition reduced cellular senescence in this cell type (Jazbutyte et al. 2013). However, its role in cardiomyocytes senescence still remains to be investigated.

miR-34a was increased in the aging mouse heart and significantly correlated with age in human heart biopsies (Boon et al. 2013). miR-34a inhibition or deletion reduced cardiomyocytes cell death and prevented cardiac contractile dysfunction in aged mice or Ku80 knockout mice, and PUNTS was identified as its target that modulated cardiac contractile function by reducing telomere shortening and DNA damage during aging (Boon et al. 2013).

miR-17-92 cluster are also aging-associated miRNAs, including six miRNAs (miR-17, miR-18a, miR-19a, miR-19b, miR-20a, and miR-92a-1) that are located

Table 3.1 miRNAs and lncRNAs in cardiac aging

Genes	Organism/organ/cell type	Function	Target	Refs.
miRNAs				
miR-22	Mouse heart tissues; neonatal rat cardiac fibroblasts	Pro-aging; pro-senescence; pro-migration;	Mimecan	Jazbutyte et al. (2013)
	Rat smooth muscle cells	Pro-senescence	Unknown	Jazbutyte et al. (2013)
miR-34a	Heart tissues of aged mouse and/or Ku80 knockout mouse	Pro-aging; pro-cardiomyocytes cell death; pro-cardiac contractile dysfunction	PUNTS	Boon et al. (2013)
miR-17-92 cluster	Mouse heart tissues; neonatal rat cardiomyocytes	Antiaging	TSP-1; CTGF	Van Almen et al. (2011)
	miR-17 transgenic mouse; heart tissues	Antiaging	Unknown	Du et al. (2015)
	Mouse cardiac fibroblasts	Anti-senescence and apoptosis; pro-epithelial-to mesenchymal transition; pro-self-renewal	Par4	Du et al. (2015)
LncRNAs				
H19	Cardiac progenitor cells	Anti-oxidative stress-induced senescence	miR-675; p53; p21; USP10	Cai et al. (2016)

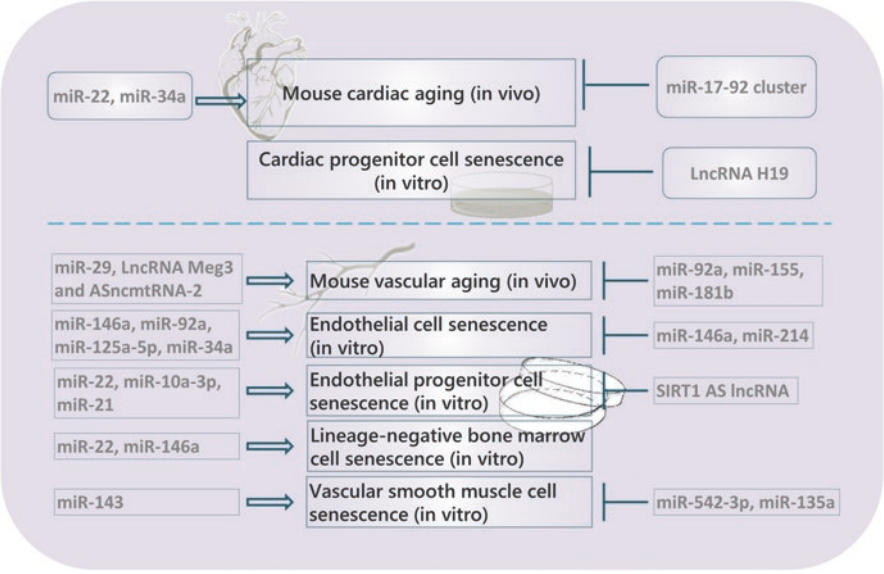

Fig. 3.1 miRNAs and lncRNAs in cardiovascular aging

on human chromosome 13 (van Almen et al. 2011). Deficient of miR-17-92 cluster severely hampered cardiac development (Ventura et al. 2008). In aged mice of C57Bl6-129Sv line (104 weeks of age), all miRNAs of miR-17-92 cluster were significantly reduced, while their targets TSP-1 and CTGF were increased in hearts as compared to 12-week littermates (van Almen et al. 2011). However, in C57Bl6 mice line, cardiac aging was accompanied by significantly increased expression of these miRNAs, except for miR-17 and miR-20a (van Almen et al. 2011). C57Bl6-129Sv and C57Bl6 mice lines have different prone to heart failure with aging, and thus the reverse changes indicate the important roles of miR-17-92 cluster in regulating aging-related heart failure. In vitro 21-day-old neonatal rat cardiomyocytes show accumulation of lipofuscin and significant induction of the thin collagen type 3A1, but not the thicker type 1A1, which is the hallmark of cardiac aging and can be used as the in vitro aging model (van Almen et al. 2011). The expression of miR-17-92 cluster was reduced significantly in cardiomyocytes cultured for 21 days compared to 4 days. Moreover, modulation of miR-18/19 changes the levels of CTGF, TSP-1, and collagens type 1A1 and 3A1 (van Almen et al. 2011). The group of Burton Yang constructed and found that miR-17 transgenic mice displayed the lower intensities of β-galactosidase staining in the heart than the wild-type mice (C57BL/6XCBA line), suggesting an inhibitory effect of miR-17 on cardiac aging (Du et al. 2015). In mouse cardiac fibroblasts, miR-17-3p promotes the epithelial-to-mesenchymal transition (EMT) and self-renewal, protects against cellular senescence and apoptosis through targeting Par4, and upregulates the downstream proteins CEBPB and FAK (Du et al. 2015).

In summary, miR-17-92 cluster have the inhibitory effects, while miR-34a and miR-22 have promotive effects on the process of cardiac aging.

3.5 MicroRNAs in Vascular Aging

Aging altered the phenotypic and functional characteristics of vasculature, contributed by the pathological changes of cells involved in the vascular system including endothelial cells, vascular smooth muscle cells, and endothelial progenitor cells. Thus, primary vascular cells isolated from old human or animals as well as multiple in vitro passaging of these cells have been used extensively as a model to study vascular aging in vitro.

The miRNA processing machinery enzyme Dicer was reported to be decreased in 2-year-old rat primary cerebral microvascular endothelial cells (CMVECs) compared with 3-month CMVECs and small cerebral vessels isolated from aged rats (Ungvari et al. 2013). miR-193b, miR-214, miR-744, miR-532, miR-672, miR-145, miR-146, and miR-574 were significantly decreased in CMVECs derived from aged animals (Ungvari et al. 2013). Increasing Dicer expression in aged CMVECs significantly increased expression of these miRNAs, whereas knockdown of Dicer in young CMVECs reduced the expression of these miRNAs, mimicking the aging phenotype (Ungvari et al. 2013). Although the detailed functions of these age-related miRNAs are not very clear, their processing enzyme Dicer was linked to the

migration rate, proliferative capacity, and adhesion to extracellular matrix of CMVECs (Ungvari et al. 2013).

To study the endothelial cell aging in human, multiple in vitro passaging of human umbilical vein endothelial cells (HUVECs), human microvascular endothelial cell line (HMEC-1), human coronary artery endothelial cells (HCAECs), and human aortic endothelial cells (HAECs) have been used as aging models. In HAECs, compared with early passage, late-passage HAECs has a senescent phenotype with reduced proliferation rate and increased staining for β-galactosidase (Rippe et al. 2012). Late-passage senescent HAECs had reduced expression of miR-21, miR-214, miR-133a, miR-126, and miR-92 and increased expression of miR-221, miR-125b, and miR-222 (Rippe et al. 2012). Using primary HUVECs as an in vitro senescence model, quantitative real-time polymerase chain reactions (qRT-PCRs) revealed that miR-16-5p, miR-28-3p, miR-106b-3p, miR-181a-5p, miR-376a-3p, and miR-886-5p were significantly deregulated (Yentrapalli et al. 2015).

In addition to the miRNA profiling studies, functional study using these human cell lines has revealed the vital roles of miR-146a, miR-34a, and miR-214 in human endothelial aging in vitro. miR-146a is a pro-inflammatory factor, and its expression in HUVECs during in vitro passaging showed conflicting results (Hackl et al. 2010; Vasa-Nicotera et al. 2011). Hackl et al. reported no significant change of miR-146a expression in HUVECs with multiple passaging (Hackl et al. 2010), while Vasa-Nicotera et al. observed a significant decrease of miR-146a in senescent HUVECs (Vasa-Nicotera et al. 2011). Contrasting results on miR-146a expression might depend on the different strains and/or normalization methods used in these two investigations. Thus, another subsequent study analyzes the expression of miR-146a not only in senescent HUVECs but also in HAECs and HCAECs (Olivieri et al. 2013). This study clearly showed that miR-146a was upregulated during endothelial cell senescence and demonstrated the association of miR-146a with senescence-associated pro-inflammatory status in vascular remodeling cells (Olivieri et al. 2013). Besides, aging-associated increase of miR-34a triggers cell senescence through suppression of SIRT1 in HUVECs and human aortic smooth muscle cells (Ito et al. 2010; Badi et al. 2015). miR-214 in exosomes suppressed cell senescence in human microvascular endothelial cell line (HMEC-1) and allowed blood vessel formation through repressing the expression of ataxia telangiectasia mutated (ATM) (van Balkom et al. 2013). Collectively, these in vitro studies indicate that miR-146a and miR-34a promote, while miR-214 suppresses human endothelial cell aging.

In mouse endothelial cells, microarray and qRT-PCR analysis on arterial endothelial cells collected from young and aging mice showed that miR-125a-5p expression was higher in old endothelial cells compared with young endothelial cells (Che et al. 2014). miR-125a-5p suppressed in vitro angiogenesis in young endothelial cells through directly repressing RTEF-1 expression and modulating eNOS and VEGF expression, resembling the aging phenotype (Che et al. 2014).

Endothelial progenitor cells (EPCs) are crucial for postnatal vascular homeostasis. The function and number of EPCs decline during aging-associated senescence. Lineage-negative bone marrow cells (lin^- BMCs) are abundant in endothelial progenitor cells and involved in vascular homeostasis and remodeling. Recently, sanger

13 microRNA array analyzes the miRNA profiles in lin⁻ BMCs from young (3-week-old) versus aged WT (2.5-year-old) mice and found that miR-10a-3p, miR-21, miR-22, and miR-146a were significantly upregulated in aged lin⁻ BMCs (Zhu et al. 2013; Deng et al. 2017). Overexpression of miR-10a-3p and miR-21 in young EPCs suppressed Hmga2 expression and caused cell senescence in vitro (Zhu et al. 2013). Inhibition of miR-146a leads to increase of Plk2 expression and functional rejuvenation of aged lin⁻ BMCs (Deng et al. 2017). Upregulation of miR-146a contributes to the impaired angiogenesis in aged mice (Deng et al. 2017). miR-22 was not only upregulated in mouse lin⁻ BMCs but also increased in aged human EPCs, and overexpression of miR-22 in young human EPCs induced cell senescence by downregulating AKT3 expression (Zheng and Xu 2014). These studies collectively indicated that miR-10a-3p, miR-21, miR-22, and miR-146a have promotive effects on vascular aging through inducing EPC senescence and subsequent cell loss.

The proliferation, migration, and transition of vascular smooth muscle cells are closely related with aging-induced neointimal formation and vascular calcification (Vazquez-Padron et al. 2004; Lin et al. 2016). miR-542-3p expression was significantly downregulated in old VSMCs isolated from 18-month rats compared with young VSMCs isolated from 8-week rats (Qian et al. 2015). Upregulation of miR-542-3p in old VSMCs significantly inhibited VSMC proliferation by directly inhibiting spleen tyrosine kinase (Syk) expression, which may explain age-related neointimal hyperplasia in rats (Qian et al. 2015). Vascular calcification is another common feature in aging population, and the transition of VSMCs toward an osteoblast-like phenotype promotes vascular calcification (Lin et al. 2016). Consecutive passage culture of VSMCs was used to determine the function of miR-135a in VSMC senescence, and miR-135a was shown to suppress calcification in senescent VSMCs by regulating KLF4/STAT3 pathway (Lin et al. 2016). In addition, miR-143 was upregulated in senescent VSMCs, and overexpression of miR-143 significantly enhanced cell senescence and reduced proliferation and migration of VSMCs (Zhao et al. 2015). Taken together, these studies implied that miR-542-3p and miR-135a suppressed the senescence of VSMCs, whereas miR-143 promoted VSMC senescence.

Compared with the numerous in vitro studies of miRNAs in vascular aging, only several miRNAs including miR-92a, miR-155, miR-181b, and miR-29 were associated with vascular aging in vivo. miR-92a is important in developmental vascular growth and significantly downregulated in arteries of aged 29-month-old B6D2F1 mice compared with young 6-month-old mice (Hazra et al. 2016). In vivo inhibition of miR-92a in young mice partially mimics the arterial aging phenotype, including reduction of nitric oxide bioavailability and endothelial-dependent dilation in response to acetylcholine as well as increase of arterial stiffness (Hazra et al. 2016). Besides, inhibition of miR-92a in young mice increases the expression of tumor necrosis factor alpha receptor (TNFR1) and type 1 collagen in the medial layer of the aortas (Hazra et al. 2016), which are reported to be important for age-related arterial dysfunction (Intengan and Schiffrin 2000). Moreover, miR-92a was also decreased in arteries of older humans (Hazra et al. 2016). However, another in vitro study revealed that miR-92a was upregulated in aged human umbilical vein

endothelial cells (Liu et al. 2017). Thus, it is abundantly clear that miR-92a suppresses mouse vascular aging, whereas the function of miR-92a in human vascular aging needs further investigation.

miR-155 is another profoundly decreased vascular miRNAs in aging mouse vessels (12 month) compared with young mice (3–4 months) (DuPont et al. 2016). And the expression of miR-155 was transcriptionally suppressed by mineralocorticoid receptors, which increases with aging and contributes to hypertension in the elderly (DuPont et al. 2016). Restoration of miR-155 in aged mice rescues the aging phenotype revealed by the attenuation of Ang II-mediated vasoconstriction and vascular oxidative stress (DuPont et al. 2016).

miR-181b was also declined in wild-type mice aorta with increase of age (4 weeks, 20 weeks, and 40 weeks) (Hori et al. 2017). There was higher systolic blood pressure in miR-181a/b knockout mice compared with WTs. Decreased miR-181b with aging plays critical roles in vascular stiffness by removing the brake on the TGF-β pathway (Hori et al. 2017).

Expression profiling of aortic tissue of young versus old mice revealed increased expression of miR-29 family members with aging (Boon et al. 2011). Aging and increased miR-29 represses the expression of extracellular matrix proteins (ECM) and make the mice aorta be sensitive to aneurysm formation in advanced age (Boon et al. 2011).

Collectively, in vivo studies demonstrated that miR-92a, miR-155, and miR-181b suppress, while miR-29 promotes the progression of vascular aging. More in-depth study based on these results will provide the potential therapeutic targets for vascular aging. A summary of miRNAs in vascular aging is given in Table 3.2 and Fig. 3.1.

3.6 LncRNAs in Cardiovascular Aging

LncRNAs are noncoding RNA transcripts that range from ~200 bases to hundreds of kilobases and do not encode proteins. The action mode of lncRNAs is dynamic, which can regulate gene transcription, pre-mRNA splicing, mRNA stability, protein translation, and protein stability (Devaux et al. 2015). Additionally, lncRNAs can interact with miRNAs and RNA-binding proteins, serving as decoys or sponges and thereby modulating the effects of these factors on target mRNAs (Devaux et al. 2015). Given the crucial roles of lncRNAs in regulating gene expression, it is not surprising that lncRNAs have been implicated in numerous biological processes such as cell proliferation, innate immunity, gene imprinting, cell apoptosis, and autophagy (Abdelmohsen and Gorospe 2015). Aberrant lncRNA expression contributes to multiple pathogenesis of human diseases including cancer, neurological disorders, and cardiovascular diseases (Abdelmohsen and Gorospe 2015). Compared with miRNAs, our understanding of lncRNA function in age-associated changes in the cardiovascular systems is much more limited.

Age-associated cardiovascular changes are at least partially ascribable to cardiac and vascular cell senescence. An mtDNA-transcribed lncRNA, ASncmtRNA-2, has

Table 3.2 miRNAs and lncRNAs in vascular aging

Genes	Organism/organ/cell type	Function in aging	Target	Ref.
miRNAs				
miR-146a	HUVECs	No function		Hackl et al. (2010)
	HUVECs	Anti-senescence	Unknown	Vasa-Nicotera et al. (2011)
	HAECs; HCAECs	Pro-senescence	Unknown	Olivieri et al. (2013)
	Mouse lin$^-$BMCs	Pro-aging; antiangiogenesis	Plk2	Deng et al. (2017)
miR-10a-3p; miR-21	EPCs	Pro-senescence	Hmga2	Zhu et al. (2013)
miR-22	Mouse lin$^-$ BMCs; human EPCs	Pro-senescence	AKT3	Zheng and Xu (2014)
miR-92a	Arteries of B6D2F1 mice and older human	Antiaging; maintain nitric oxide bioavailability; inhibit arterial stiffness	TNFR1 and type 1 collagen	Hazra et al. (2016)
	HUVECs	Pro-aging	Unknown	Liu et al. (2017)
miR-34a	HUVECs; human aortic smooth muscle cells	Pro-senescence	SIRT1	Ito et al. 2010; Badi et al. (2015)
miR-542-3p	Primary rat VSMCs	Antiaging; anti- proliferation	Syk	Qian et al. (2015)
miR-135a	VSMCs	Antiaging; anti-vascular calcification	KLF4/STAT3 pathway	Lin et al. (2016)
miR-143	VSMCs	Pro-senescence; anti-proliferation and migration	Unknown	Zhao et al. (2015)
miR-214	Human microvascular endothelial cell line	Anti-senescence; pro-blood vessel formation	ATM	Van Balkom et al. (2013)
miR-29	Mouse aortic tissue	Pro-aging	Extracellular matrix proteins	Boon et al. (2011)
miR-155	Mouse vessels	Antiaging; reduce AngII-mediated vasoconstriction and vascular oxidative stress	Unknown	DuPont et al. (2016)
miR-181b	miR-181a/b knockout mice	Antiaging; anti-systolic blood pressure; anti-vascular stiffness	Unknown	Hori et al. (2017)

(continued)

Table 3.2 (continued)

Genes	Organism/organ/cell type	Function in aging	Target	Ref.
miR-125a-5p	Primary mouse arterial endothelial cells	Pro-aging; antiangiogenesis	RTEF-1	Che et al. (2014)
LncRNAs				
ASncmtRNA-2	Mouse aortas; endothelial cells	Pro-aging; anti-proliferation	Hsa-miR-4485; hsa-miR-1973	Bianchessi et al. (2015)
Meg3	HUVECs; human cardiac atria samples; mouse blood vessels	Pro-aging; anti-sprouting activity of HUVECs; reduce the perfused vessels after ischemic	Unknown	Boon et al. (2016)
SIRT1 AS lncRNA	Mouse EPCs	Anti-senescence; pro-proliferation; and migration	miR-22	Ming et al. (2016b)

HUVECs, human umbilical vein endothelial cells; *HAECs,* human aortic endothelial cells; *HCAECs,* human coronary artery endothelial cells; *EPCs,* endothelial progenitor cells; *lin⁻BMCs*, lineage-negative bone marrow cells

been shown increased in aortas of old mice (Bianchessi et al. 2015). In vitro analysis established that ASncmtRNA-2 is induced in endothelial cells during senescence but not in vascular smooth muscle cells, imitating the expression pattern of aging-associated gene p16 (Bianchessi et al. 2015). Transiently overexpressing ASncmtRNA-2 in endothelial cells caused the cell cycle arrest in G2/M phase during cell senescence, possibly through the production of miR-4485 and miR-1973 which are homologous to the double-strand region of ASncmtRNA-2 (Bianchessi et al. 2015).

LncRNA Meg3 was significantly increased lncRNA in senescent HUVECs compared with early passage HUVECs (Boon et al. 2016). Importantly, Meg3 was strongly correlated with age in human cardiac atria samples. In vitro, silencing of Meg3 in HUVECs prevented aging-mediated inhibition of sprouting activity HUVECs (Boon et al. 2016). And in vivo, reducing Meg3 expression significantly improved the blood flow and increased the perfused vessels in ischemic mouse legs (Boon et al. 2016). Although the in vivo roles of Meg3 in vascular aging remain to be elucidated, this study highlighted Meg3 inhibition as a potential therapeutic strategy to rescue endothelial dysfunction in aging.

Numerous studies have implicated the importance of endothelial progenitor cells in cardiovascular aging. Nicotinamide phosphoribosyltransferase (NAMPT) reduced the decrease of β-galactosidase activity and increased the telomerase activity in EPCs, thereby inhibiting EPC senescence and promoting angiogenesis (Xiao et al. 2009). Further studies demonstrated that SIRT1 AS lncRNA, a natural antisense transcript of SIRT1, was elevated by NAMPT in mouse EPCs and contributed to the inhibition of cell senescence as well as the promotion of proliferation and migration of EPCs (Ming et al. 2016a, b). Mechanically, SIRT1 AS lncRNA served

as miR-22 sponge, and overexpression of SIRT1 AS lncRNA attenuated the miR-22-induced downregulation of SIRT1 in EPCs (Ming et al. 2016b). Moreover, SIRT1 AS lncRNA also counteracted the downregulation of SIRT1 induced by miR-34a in C2C12 cells (Wang et al. 2014). miR-22, miR-34a, and SIRT1 have been shown crucial for cardiovascular aging (Ming et al. 2016a; Zheng and Xu 2014; Badi et al. 2015; Ito et al. 2010; Boon et al. 2013; Jazbutyte et al. 2013); thereby the in vivo roles of SIRT1 AS lncRNA in cardiovascular aging highly deserved to be investigated in mouse and/or human.

The senescence of cardiac progenitor cells (CPCs) is responsible for the decline of CPCs' functions and regenerative potential during cardiac aging. As one of the most well-studied lncRNAs, lncRNA H19 is a crucial regulator of cellular proliferation, senescence, fibrosis, etc. (Ratajczak 2012). Exon 1 of lncRNA H19 carries the template for miR-675, which has been shown responsible for the H19-regulated cellular processes (Keniry et al. 2012). Interestingly, lncRNA H19 and its derived miR-675 were downregulated during oxidative stress-induced senescence of CPCs (Cai et al. 2016). H19 knockdown promoted, while miR-675 overexpression inhibited the upregulation of p53 and p21 proteins as well as the premature cell senescence induced by oxidative stress in CPCs (Cai et al. 2016). Furthermore, USP10 was identified as the target of H19-derived miR-675, and USP10 was responsible for the downregulation of p53 and p21 proteins after miR-675 overexpression (Cai et al. 2016). H19 has been identified as an important regulator of cardiac hypertrophy and cardiac fibrosis (Tao et al. 2016; Liu et al. 2016; Huang et al. 2017), which are common diseases in elder population. Thus, it is worthy to further investigate whether H19 regulates cardiac aging in vivo.

To our best knowledge, in vivo roles and mechanisms of lncRNA in cardiac and vascular aging are lacking. In addition to abovementioned lncRNAs, the profiling study of lncRNAs provides valuable evidences for further functional study in cardiovascular aging. A study performed RNA sequencing to analyze the RNA transcript abundance in plasma endothelial vesicles isolated from 50 healthy controls and 142 cancer patients (range 25–79 years old). Within the 50 healthy controls, three lncRNAs (EHHADH-AS1, RP11-696 N14.1, and AC022311.1) were found to be positively associated with increased age (Yuan et al. 2016). RNA deep sequencing has identified LINC00657, TUG1, Meg3, MALAT1, and LINC00493 as the five highest expressed conserved lncRNAs in HUVECs (Boon et al. 2016). Another RNA-sequencing study revealed that LncRNA SENCR (smooth muscle and endothelial cell-enriched migration/differentiation-associated long noncoding RNA) was selectively enriched in human coronary artery smooth muscle cells and HUVECs (Bell et al. 2014). In addition, several studies have demonstrated the importance of lncRNAs in aging-related cardiovascular diseases such as coronary heart disease, atherosclerosis, hypertension, and heart failure. The mitochondrial lncRNA LIPCAR is upregulated during later stages following myocardial infarction, and its plasma level may predict survival in patients with heart failure (Kumarswamy et al. 2014; Dorn 2014). LncRNA ANRIL have been shown to influence the susceptibility to coronary heart disease (McPherson et al. 2007). Another study revealed 15 lncRNAs were altered in murine failing hearts (Lee et al. 2011).

These studies also showed the deserving hints for investigation of lncRNAs in cardiovascular aging. A summary of the roles of lncRNAs in cardiac aging and vascular aging was given in Tables 3.1 and 3.2 and Fig. 3.1, respectively.

3.7 Conclusions

The elderly population is increasing year by year, and it is still lack of efficient interventions to attenuate or reverse the negative physiological consequences and the concomitant aging-related cardiovascular dysfunction in the elderly. Thus, there is an urgent need to disclose the underlying mechanisms of cardiovascular aging. As we reviewed here, numerous studies have demonstrated that miRNAs and lncRNAs played major roles in cardiovascular aging. However, compared with their large quantity and variety, only a few miRNAs and lncRNAs are identified crucial for cardiovascular aging. In particular, knowledge about the in vivo functions of miRNAs and lncRNAs in cardiovascular aging are still very limited. Besides, other ncRNAs such as circular RNAs have recently emerged as vital regulators of cardiovascular diseases, but no circular RNAs have been implicated in cardiovascular aging. Future studies are necessary and crucial to identify more important ncRNAs in regulating cardiovascular aging, which will provide more in-depth understanding of intrinsic cardiovascular aging and gain more potential therapeutic targets to combat aging-related cardiovascular diseases.

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Epigenetic Regulation of Vascular Aging and Age-Related Vascular Diseases

4

Yang-Nan Ding, Xiaoqiang Tang, Hou-Zao Chen, and De-Pei Liu

Abstract

Vascular aging refers to the structural and functional defects that occur in the aorta during the aging process and is characterized by increased vascular cell senescence, vascular dyshomeostasis, and vascular remodeling. Vascular aging is a major risk factor for vascular diseases. However, the current understanding of the biological process of vascular aging and age-related diseases is insufficient. Epigenetic regulation can influence gene expression independently of the gene sequence and mainly includes DNA methylation, histone modifications, and RNA-based gene regulation. Epigenetic regulation plays important roles in many physiological and pathophysiological processes and may explain some gaps in our knowledge regarding the interaction between genes and diseases. In this review, we summarize recent advances in the understanding of the epigenetic regulation of vascular aging and age-related diseases in terms of vascular cell senescence, vascular dyshomeostasis, and vascular remodeling. Moreover, the possibility of targeting epigenetic regulation to delay vascular aging and treat age-related vascular diseases is also discussed.

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Epigenetic regulation · Vascular aging · Cell senescence · Vascular dyshomeostasis · Vascular remodeling

4.1 Introduction

Cardiovascular diseases (CVDs) are the leading cause of death worldwide (Leong et al. 2017). Vascular diseases (VDs), such as atherosclerosis and abdominal aortic aneurysm (AAA), account for a large proportion of CVDs. Advanced age is the most significant independent risk factor for future vascular events. The incidence of VDs continues to increase because of the increase in the aging population.

As early as 1680, Dr. Thomas Sydenham proposed that "you are as old as your arteries." This old aphorism emphasizes the importance of vascular aging, which is considered a determining factor of VDs and health status in the elderly (El Assar et al. 2013). For centuries, exploring the secrets of vascular aging in terms of both the structural and functional defects that occur in the aortas has been of great interest. At the macroscopic level, aged vessels are remodeled and characterized by luminal dilation, diffused stiffness, and arterial wall thickening. At the microscopic level, aged vessels are characterized by increased vascular cell senescence and an imbalance in vascular homeostasis (Fig. 4.1). Vascular cell senescence, vascular dyshomeostasis, and vascular remodeling are the three characteristics of vascular aging.

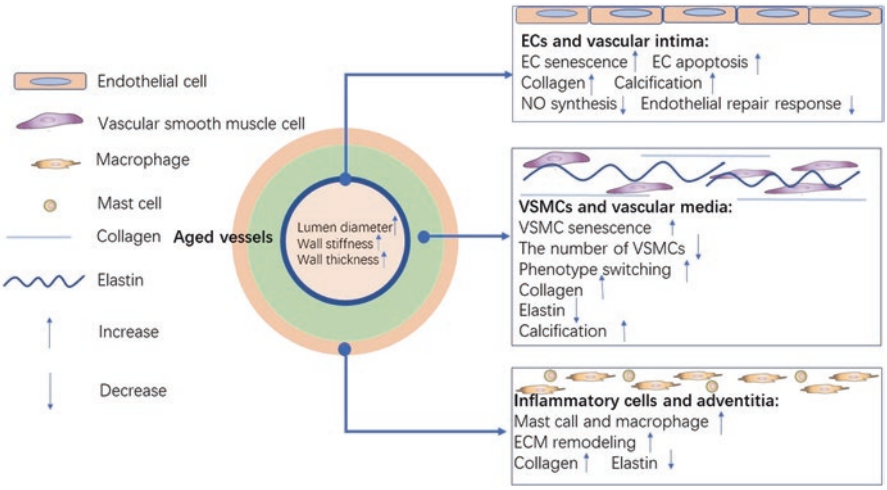

Fig. 4.1 The changes of aged vessels. Compared to normal vessels, aged vessels are characterized by increased lumen diameter, wall stiffness, and thickness. As shown in the picture, the cells and extracellular matrix undergo structural and functional changes at the same time. *EC* endothelial cell, *VSMC* vascular smooth muscle cell, *ECM* extracellular matrix, *NO* nitric oxide

The risk factors for vascular aging and VDs, including older age, sedentariness, cigarette smoking, and high-salt and high-fat diet intake, are closely linked to epigenetic regulation, which play key roles in the association between gene expression and environmental insults. Accumulating evidence has demonstrated that epigenetic factors are critically involved in age-related vascular diseases (Grimaldi et al. 2015; Kim and Stansfield 2017). In this review, we summarize the role of epigenetic regulation in vascular aging.

4.2 Epigenetic Regulation of Vascular Cell Senescence

4.2.1 Changes in Vascular Cells During the Vascular Aging Process

Endothelial cells (ECs) and vascular smooth muscle cells (VSMCs), which are the two most important types of vascular cells, are considered as the embodiment and executors of vascular aging. Normally, ECs contact the blood directly and play key roles in regulating arterial functions, including vascular tone, vascular permeability, angiogenesis, and the response to inflammation. VSMCs embed in the extracellular matrix (ECM), display remarkable phenotypic plasticity, and can establish, maintain, and repair the aortic media (Safar 2010). Remarkable defects in the structure and function of ECs and VSMCs have been discovered in aged aortas.

ECs from older donors are fattened and enlarged and display endothelial dysfunction and regenerative disability. These defects are accompanied by reduced nitric oxide (NO) synthesis and increased endothelin-1 expression. In addition, the endothelial repair response is impaired in aged arteries (Haendeler et al. 2004; Mirea et al. 2012; Wang and Bennett 2012). VSMCs derived from aged vessels show features of cellular senescence, including reduced proliferation and a shift from a contractile to synthetic phenotype (Owens et al. 2004; Bennett et al. 2016). This phenotypic switching contributes to the increased stiffness of the aortas and dilatation of the conduit arteries, which are the hallmarks of vascular aging (Osborne-Pellegrin et al. 2010).

4.2.2 Epigenetic Regulation of Vascular Cell Senescence

Cell senescence, which is an irreversible state of cell proliferative arrest, is a hallmark of aging and an important contributor to age-related diseases (Childs et al. 2015). A variety of senescence stressors can lead to cell senescence by affecting gene expression, as summarized in earlier reviews (Munoz-Espin and Serrano 2014; He and Sharpless 2017). Importantly, recent advances have enriched our understanding of epigenetic mechanisms in cell senescence during vascular aging.

SIRT1, which is a member of NAD^+-dependent class III histone deacetylase (HDAC) family, has drawn great attention due to its anti-senescent effect in vascular aging. The overexpression of SIRT1 can delay the progression of human umbilical

vein endothelial cell (HUVEC) senescence caused by hydrogen peroxide (Ota et al. 2007). The results from our laboratory showed that SIRT1 could decrease vascular endothelial replicative senescence in vitro and improve EC function in vivo by binding the *PAI-1* promoter and decreasing the histone H4 lysine 16 (H4K16) acetylation level (Wan et al. 2014). We also showed that the loss of SIRT1 in aged aortas increased angiotensin (Ang)-II-induced VSMC senescence, upregulated *p21* expression, and enhanced inflammatory cell recruitment (Chen et al. 2016). In addition to the well-established anti-senescence roles of SIRT1, the functions of other HDACs in the senescence and dysfunction of vascular cells and vascular aging have also been reported (Table 4.1).

Furthermore, vascular cell senescence is regulated by miRNAs, such as miR-217. MiR-217 is progressively expressed in ECs with aging and inhibits SIRT1 expression by binding the 3′-UTR of *SIRT1*. The knockdown of miR-217 in ECs delayed cell senescence, whereas miR-217 overexpression resulted in an early senescent-like phenotype in young ECs (Menghini et al. 2009). Related information about miRNAs and cell senescence has been summarized in earlier reviews (Menghini et al. 2014; Araldi and Suarez 2016).

DNA methylation, especially age-dependent promoter methylation, has been proposed as a possible epigenetic mechanism underlying age-related vascular diseases (Toghill et al. 2015; Hai and Zuo 2016). However, knowledge regarding the effect of DNA methylation on vascular cell senescence is limited. Further work is needed to elucidate the potential roles of DNA modification in vascular aging.

Table 4.1 HDACs and vascular cells

	HDACs	Functions	Targets	References
Class I HDACs	HDAC2	Modulate EC function	Arginase 2 and eNOS	Pandey et al. (2014, 2015)
	HDAC3	Maintain EC survival	Akt activation	Zampetaki et al. (2010)
	HDAC4	SMC proliferation and migration		Usui et al. (2014)
Class II HDACs	HDAC5	Repress EC migration and sprouting	FGF2	Urbich et al. (2009)
		Repress EC growth	FGF2 and Slit2	Urbich et al. (2009)
		AngII-mediated VSMC gene transcription	Src-PLCγ-CamK II-HDAC5 signaling pathway	Pang et al. (2008)
	HDAC7	EC growth	β-catenin	Margariti et al. (2010)
		EC cycle and growth	MMP10	Chang et al. (2006)
		EC migration	FGF2	Urbich et al. (2009)
	HDAC9	Promote EC migration	FGF2	Urbich et al. (2009)

(continued)

Table 4.1 (continued)

	HDACs	Functions	Targets	References
Class III HDACs	SIRT1	Prevent EC apoptosis and senescence	Akt1 and FoxO3a	Hou et al. (2010)
		Promote EC proliferation	Serine/threonine kinase and LKB1	Zu et al. (2010)
		Prevent EC senescence	p53-p21-pathway	Warboys et al. (2014)
		Prevent EC autophagy	ROS-elicited autophagy	Liu et al. (2015)
		Maintain EC vasoprotective phenotype	KLF2	Gracia-Sancho et al. (2010)
		Improve EC function	PAI-1	Wan et al. (2014)
		Inhibit VSMC apoptosis		Mathew et al. (2010)
	SIRT3	Promote EC antioxidant effect	H2S	Xie et al. (2016)
		Protect ECs from oxidative stress	SOD2	Winnik et al. (2016)
	SIRT6	Inhibit EC senescence and inflammation	ROS-mediated posttranslational modification, NF-κB	Hu et al. (2015) and Balestrieri et al. (2015)

In summary, epigenetic factors, such as SIRT1, can directly inhibit vascular cell senescence. Many epigenetic regulators are involved in the function of vascular cells, but whether these regulators influence cell senescence and vascular aging remains unknown.

4.3 Epigenetic Regulation of Age-Associated Vascular Dyshomeostasis

Vascular homeostasis refers to a healthy and balanced physiological state in which the vascular cells coordinate with the environment to maintain normal functioning in the aortas. For example, vascular homeostasis is essential for maintaining an appropriate blood pressure and proper tissue blood perfusion (Liu et al. 2014). Vascular cells are the determinants of vascular homeostasis. Although aging organisms do not always show high levels of cell senescence, senescent cells create a microenvironment that facilitates the initiation and progression of cell dysfunction, which may destroy vascular homeostasis (Chen et al. 2016; Cheng et al. 2017). Oxidative stress, calcification, and inflammation are the most important pathophysiological processes of vascular dyshomeostasis during aging (Ji et al. 1998; Pitocco et al. 2013).

4.3.1 Oxidative Stress

Oxidative stress, which represents an imbalance between the oxidative and antioxidative systems, is a major feature of vascular aging. Aged vessels generate excessive reactive oxygen species (ROS), such as superoxide anions (O_2^-), hydroxyl radicals (OH), and hydrogen peroxide (H_2O_2), which affect antioxidants such as nitric oxide (NO) and superoxide dismutases (SODs) (Finkel 2003).

Aged ECs exhibit a decrease in NO production because of the decreased expression of endothelial nitric oxide synthase (eNOS) (Versari et al. 2009; Cau et al. 2012). Under physiological conditions, the promoter of *NOS3*, which encodes eNOS, is hypomethylated, whereas *NOS3* promoter hypermethylation was observed under pathological conditions (Chan et al. 2004). The *NOS3* promoter in ECs also has multiple histone modifications, including acetylated H3K9, acetylated H4K12, and di- and trimethylated H3K4 (Xu 2014). For example, increased H3 and H4 acetylation across the *NOS3* promoter region (−4501/+23) upregulated the transcription of *NOS3* (Xu et al. 2010). Furthermore, SIRT1 activated eNOS and promoted NO production by deacetylating eNOS on Lys496 and Lys506 (Mattagajasingh et al. 2007). Inhibiting SIRT1 by RNAi or pharmacologic methods decreased eNOS expression, while endothelium-specific overexpression of SIRT1 upregulated eNOS expression (Ota et al. 2007; Zhang et al. 2008).

The reduced vascular NO production and availability could increase ROS generation in human vessels, and mitochondria account for most cellular ROS production during the aging process (Guzik et al. 2002; van der Loo et al. 2009). SIRT3, which is a member of the class III HDAC family, is located in the mitochondrion and maintains cellular ROS levels by deacetylating and activating several enzymes. SIRT3 deficiency led to the hyperacetylation and inactivation of the antioxidant superoxide dismutase 2 (SOD2), which resulted in the accumulation of mitochondrial O_2^- (Dikalova et al. 2017). Moreover, $p66^{Shc}$ is a vital redox enzyme implicated in mitochondrial ROS production that can mediate electron transfer from reduced cytochrome *c* to H_2O_2 (Giorgio et al. 2005). SIRT1 decreases mitochondrial ROS generation by inhibiting $p66^{Shc}$ expression (Zhou et al. 2011). Notably, the promoter of $p66^{Shc}$ contains a high content of CpG islands, and the results based on different cell lines have shown a strong correlation between promoter methylation and the expression of $p66^{Shc}$ (Ventura et al. 2002; Cencioni et al. 2013). MiR-210 also modulates mitochondrial ROS production and sensitivity by disturbing the normal function of mitochondrial compounds. For example, miR-210 negatively regulates cytochrome c oxidase assembly homolog 10 (*COX10*), and the loss of COX10 inhibits the activity of mitochondrial complex I and complex IV (Chan and Loscalzo 2010; Chen et al. 2010; Cencioni et al. 2013; Magenta et al. 2013).

4.3.2 Calcification

Vascular calcification is another feature of aged vessels. Usually, vascular calcification is observed as intimal plaques or is located at the level of elastic fibers within the media (Paneni et al. 2015; Wu et al. 2015; Janzen and Vuong 2001). Vascular calcification is mostly due to changes in VSMCs. Replicative senescence, osteogenic transdifferentiation, disturbed Ca^{2+} efflux in VSMCs, and a high blood phosphate concentration lead to vascular calcification during the aging process (Kapustin and Shanahan 2016).

Senescent VSMCs are more sensitive to phosphate-induced calcification than young VSMCs. Resveratrol-mediated SIRT1 activation can significantly reduce senescence-associated calcification (Takemura et al. 2011). In addition, a high phosphate concentration increased DNA methyltransferase (DNMT) activity and the methylation of the promoter region of *SM22α*, which reduced SM22α expression and increased the alkaline phosphatase level, leading to calcification in vitro (Montes de Oca et al. 2010). Moreover, many miRNAs, such as miR-135a, miR-762, miR-714, and miR-712, can promote vascular calcification by disrupting Ca^{2+} efflux proteins in VSMCs, including sodium/calcium exchange protein 1 (NCX1) and ATPase plasma membrane Ca^{2+} transporting 1 (PMCA1) (Gui et al. 2012). In contrast, some miRNAs inhibit vascular calcification. For example, miR-125b inhibits VSMC osteogenic transdifferentiation by targeting osteoblast transcription factor *SP7* in vivo and in vitro (Goettsch et al. 2011), and the overexpression of miR-125b inhibits β-glycerophosphoric acid-induced osteogenic marker expression by targeting *Ets1* (Wen et al. 2014). Additional information about miRNAs and vascular calcification is provided in Table 4.2.

4.3.3 Inflammation

Inflammation is another hallmark of vascular aging (Humphrey and Milewicz 2017). Low-grade chronic inflammation is a common feature of aging tissues, even in the absence of acute or other physiological stress. Inflammation is partly due to systemic inflammation during aging and also derives from changes in local cells (Singh and Newman 2011; Childs et al. 2017). Due to their senescent state, ECs and VSMCs are more closely related to inflammatory cells in the aortas (Wang and Bennett 2012). Additionally, accumulating evidence has shown that inflammatory cells, such as macrophages and monocytes, exist in the aortic wall and contribute to the development of age-related vascular diseases (2017; Jia et al. 2017). In addition, pro-inflammatory transcription factors, such as nuclear factor κB (NF-κB), which regulates the expression of multiple inflammatory factors, are also activated during the aging process (El Assar et al. 2013) and are also regulated by epigenetic factors.

The DNA methylation status links the risk and severity of chronic inflammatory conditions. A global genome-wide methylation study in humans demonstrated that global DNA hypermethylation was associated with inflammation in age-related diseases (Cutolo et al. 2014). Furthermore, promoter hypomethylation of monocyte

Table 4.2 MiRNAs and vascular calcification

Function	MiRNAs	Mechanism	Targets	References
Inhibit vascular calcification	MiR-125b	Inhibit VSMC osteogenic transdifferentiation	Osteoblast transcription factor SP7	Goettsch et al. (2011)
		Inhibit osteogenic marker expression and calcification	Ets1	Wen et al. (2014)
	MiR-133a	Inhibit VSMC osteogenic transdifferentiation	Alkaline phosphatase activity, osteocalcin, RUNX2	Liao et al. (2013)
	MiR-133b	Inhibit high phosphorus-induced VSMC calcification	RUNX2	Panizo et al. (2016)
	MiR-211			
	MiR-204	Attenuate vitamin D3-induced medial artery calcification	RUNX2	Cui et al. (2012)
	MiR-34b/c	Inhibit aldosterone-induced VSMC calcification	SATB2/RUNX2 pathway	Hao et al. (2016)
	MiR-297a	Inhibit vitamin D3 plus nicotine-induced vascular calcification	FGF23-Klotho axis	Zheng et al. (2016)
	MiR-135a	Suppress calcification in senescent VSMCs	KLF4/STAT3 pathway	Lin et al. (2016)
	MiR-29b-3p	Inhibit vitamin D3 plus nicotine- induced vascular calcification	MMP2	Jiang et al. (2017)
Promote vascular calcification	MiR-32	Promote VSMC calcification	Bone morphogenetic protein-2, RUNX2, osteopontin, bone-specific phosphoprotein matrix GLA protein	Liu et al. (2017)
	MiR-29b	Promote high phosphorus-induced VSMC calcification	Activin A receptor type IIA, β-catenin-interacting protein 1	Panizo et al. (2016)
	MiR-29	Phosphorus-induced VSMC osteoblastic differentiation	Elastin	Sudo et al. (2015)
	MiR-135a	Promote phosphate and calcium-induced VSMC calcification by disrupting Ca^{2+} efflux	Sodium/calcium exchange member 1, plasma membrane calcium pump isoform 1, sodium/potassium/calcium exchange member 4	Gui et al. (2012)
	MiR-762			
	MiR-714			
	MiR-712			
	MiR-211/222 family	Influence cellular inorganic phosphate and pyrophosphate levels	RUNX2 and MSX2, ectonucleotide phosphodiesterase 1, Pit-1	Mackenzie et al. (2014)
	MiR-205	β-glycerophosphate-induced calcification of HASMCs	RUNX2 and SMAD1	Qiao et al. (2014)

chemoattractant protein-1 (*Mcp-1*) may facilitate the development of atherosclerosis (Liu et al. 2012). In addition, DNA methylation of the *Foxp3* promoter influenced $CD4^+$ Th1/Th2 differentiation, thereby playing a protective role in experimental atherosclerosis (Jia et al. 2013). Human monocytes exposed to oxidized low-density lipoprotein (ox-LDL) for 24 h could enrich H3K4me3 on the promoter of IL-6, IL-18, MCP-1, and TNF-α, thus accelerating the formation of foam cells (Bekkering et al. 2014).

HDACs also affect the inflammatory response. For example, SIRT6 deacetylates H3K9 and H3K56 to regulate the expression of killer cell lectin-like receptor K1 (*Klrk1*, *NKG2D*) ligands and subsequently repress atherosclerosis in mice (Zhang et al. 2016). HDAC3 plays a major role in the activation of inflammatory gene expression in macrophages. Macrophages lacking HDAC3 are hyperresponsive to interleukin-4, which drives macrophages toward a specific polarized state known as alternative activation (Mullican et al. 2011). Importantly, HDACs regulate gene expression not only by histone acetylation but also through the acetylation of transcription factors (Choudhary et al. 2009). SIRT1 can reduce COX-2 expression by deacetylating and inhibiting transcription factor activator protein-1 (*AP-1*) in macrophages (Zhang et al. 2010). Moreover, HDAC inhibitors increase the expression of the NF-κB-downstream molecule interleukin 8, whereas overexpression of HDAC1 and HDAC2 represses its related gene expression (Ashburner et al. 2001).

Many miRNAs participate in vascular inflammation, and many miRNAs perform opposing functions (Grimaldi et al. 2015). For example, miR-155 is a positive regulator of vascular inflammation, and the overexpression of miR-155 results in a chronic inflammatory condition (O'Connell et al. 2012). Evidence shows that miR-155 is highly expressed in activated macrophages and ox-LDL stimulated monocytes. The high level of miR-155 in macrophages boosts the expression of MCP-1, which recruits additional monocytes to dysfunctional ECs (Nazari-Jahantigh et al. 2012). Moreover, elevated miR-155 enhances ox-LDL-induced foam cell formation by directly repressing the expression of HMG box-transcription protein1 (HBP1) (Tian et al. 2014). Furthermore, miR-155 promotes inflammatory transcription factors by repressing the expression of suppressor of cytokine signaling-1 *(SOCS1)* and B-cell lymphoma 6 (*BCL6*), which attenuate NF-κB signaling (Nazari-Jahantigh et al. 2012, Yang et al. 2015). In contrast, miR-194 and miR-223 are negative regulators of inflammation. The overexpression of miR-194 decreases tumor necrosis factor receptor-associated factor 6 (TRAF6) expression and attenuates the release of the pro-inflammatory cytokine TNF-α in monocytes (Tian et al. 2015). MiR-223 negatively regulates the activation of the toll-like receptor 4 (TLR4)-NF-κB pathway by activating the PI3/AKT pathway, and increasing miR-223 can significantly attenuate macrophage foam cell formation, lipid accumulation, and pro-inflammatory cytokine production (Zhang et al. 2014; Wang et al. 2015).

4.3.4 Cross Talk Between Vascular Cells and Vascular Dyshomeostasis

As a result of vascular cell senescence and dysfunction, vascular dyshomeostasis is a major pathophysiological change that occurs during aging. More importantly, vascular dyshomeostasis, in turn, promotes cell senescence and dysfunction. For example, ROS promotes EC senescence by inhibiting telomerase- or telomere-independent mechanisms, and ROS-related signaling participates in EC apoptosis and endothelium injury (Erusalimsky 2009; El Assar et al. 2013). ROS also mediates many pathophysiological processes in VSMCs, including growth, migration, differentiation, and the secretion of inflammatory cytokines (Clempus and Griendling 2006).

A complex association also exists between inflammation and oxidative stress. Excessive or uncontrolled free radical production can induce an inflammatory response, and free radicals are also inflammation effectors. In contrast, the redox-sensitive transcription factor NF-κB is activated in vascular cells from elderly subjects and drives a pro-inflammatory shift that feeds back on oxidative stress. NF-κB mediates the H_2O_2-induced response in vascular cells, and NF-κB inhibition reduces the expression of NADPH oxidase, which is another major source of ROS in the vasculature in overweight/obese middle-aged and older human (Ago et al. 2004; Pierce et al. 2009).

Given the complexity and diversity of vascular dyshomeostasis, knowledge regarding the epigenetic regulation of vascular homeostasis is highly insufficient. More studies are needed to elucidate the role of epigenetic regulation in the maintenance of vascular homeostasis.

4.4 Epigenetic Regulation of Age-Associated Vascular Remodeling

Vascular remodeling, which is the most intuitive feature of vascular aging, directly induces defects in vascular function. Unresolved vascular remodeling may be a key element in virtually all vascular diseases (Schwartz et al. 2018). Epidemiological, cross-sectional, clinical, and postmortem studies have demonstrated that intima and media wall thickening occur in arteries with advanced age. This change is evolutionarily conserved in various species, including rats, rabbits, and nonhuman primates (Wang et al. 2010).

Elastin and collagen fibers are the major components of the ECM; with increasing age, the elastin content decreases, whereas the synthesis and deposition of collagen increase (Mauriello et al. 1992; Najjar et al. 2005). Elastin expression is regulated by miRNAs from the miR-15 family (especially miR-195) and miR-29 family (especially miR-29b) (Maurice et al. 2013). Many CpG islands are present near the transcriptional start sites of many collagen genes, suggesting that DNA methylation plays an important role in regulating collagens (Chernov and Strongin 2011). For instance, the transcript and protein levels of collagen, type XV, alpha 1 (*COL15A1*) increase with passage-dependent decreases in DNA methylation

(Connelly et al. 2013). Furthermore, our results have demonstrated that SIRT1 could suppress the upregulation of collagen induced by AngII (Gao et al. 2014).

Matrix metalloproteinases (MMPs) constitute a family of zinc-dependent endopeptidases that degrade and deposit structural proteins within the ECM. The increased production and activation of MMPs, especially MMP-2 and MMP-9, are hallmark features of vascular remodeling in aged vessels. Moreover, endogenous tissue inhibitors of metalloproteinases (TIMPs) reduce excessive proteolytic ECM degradation by the MMPs. An imbalance in MMP/TIMP activity plays a major role in age-related vascular remodeling (Amin et al. 2016). Both MMPs and TIMPs are under the control of epigenetic factors.

For instance, an inverse correlation has been observed between the level of methylation of CpG nucleotides located at the *MMP-9* promoter and constitutive MMP-9 expression, and the methylation of the *MMP-9* promoter abolished its transcriptional activity. Selective class I HDAC inhibitors reduce the expression and activity of both MMP-2 and MMP-9 and attenuate ECM remodeling (Mani et al. 2015). Moreover, many miRNAs participate in the regulation of the MMPs. For example, miR-106a inhibits MMP-2 expression by modulating the transcription factor Ets-1 (Shin et al. 2016). Another report showed that the age-associated increase in miR-29b upregulates the activity of MMP-2/MMP-9 and that inhibiting miR-29b could decrease the expression of MMP-9 in the aorta (Boon et al. 2011; Merk et al. 2012). In contrast, a reduction in miR-195 is associated with the upregulation of MMP-2/MMP-9 at the gene and protein level, promoting vascular remodeling in mouse models (Zampetaki et al. 2014).

In addition, promoter methylation regulated the expression of tissue factor pathway inhibitor-2 (*TFPI-2*), which inhibited a wide variety of serine proteinases that participate in ECM degradation. TFPI-2 gene hypermethylation was observed in aging vascular tissues and approximately 30% of atherosclerotic plaques (Zawadzki et al. 2009). Moreover, miR-181b negatively regulates TIMP3 expression and elastin production and greatly increases elastin and collagen expression (Di Gregoli et al. 2017).

4.5 Age-Related Vascular Diseases

Aging is among the key risk factors for vascular diseases, including AAA, atherosclerosis, and diabetic vascular dysfunction. Epidemiological studies have shown that AAAs are found in up to 8% of men aged over 65 years, and the risk of AAA increases by 40% every 5 years after the age of 65 (Nordon et al. 2011). Atherosclerosis is another common vascular disease in the elderly, and aging is an important risk factor and an independent contributor to atherosclerosis (Wang and Bennett 2012). The incidence of diabetes-related vascular complications and stroke also increases with age (Ly and Maquet 2014, Keating et al. 2016). This increased complication rate is not only due to the increase in disease-related risk factors but also because of the direct effect of aging on blood vessels perse.

Cell senescence, vascular dyshomeostasis, and vascular remodeling are typical pathophysiological changes in vascular diseases. However, the following quandary exists in the relationship between vascular diseases and vascular aging: does aging lead to diseases, or do diseases drive vascular aging? The hypothesis that a bidirectional relationship exists between diseases and aging is more reasonable than a one-way relationship. Both aging and disease could occur simultaneously and form a vicious circle. Because of the similarity and simultaneity between vascular aging and age-related vascular diseases, both may share the same epigenetic changes. Epigenetic-related methods that delay the vascular aging process may be a potential treatment for diverse vascular diseases.

4.6 Epigenetic-Targeted Therapeutic Strategies for the Treatment of Vascular Aging and Age-Related Vascular Diseases

Preventing or delaying vascular aging and age-related diseases is a critical goal for reducing morbidity, disability, and health-care costs. Because of the complexity of vascular aging, delaying the aging process by targeting one type of cell or pathological change is difficult. Since epigenetic regulation participates in all aspects of vascular aging, developing new targeted therapies that slow or even abolish some of the consequences of vascular aging by epigenetic regulation could be highly valuable (Mirea et al. 2012).

SIRT1 dysregulation is involved in various aspects of vascular aging; therefore, SIRT1 is a promising epigenetic target for delaying vascular aging and inhibiting age-related vascular diseases. Resveratrol, which is a polyphenol found in red wines, is a classic natural SIRT1 activator and has been demonstrated to delay vascular aging in rat and mouse models (da Luz et al. 2012; Kim et al. 2018). Small-molecule SIRT1 activators, such as SRT2104 and SRT1720, have been shown to improve health and extend the lifespan in mice (Minor et al. 2011; Mercken et al. 2014). Notably, SRT2104 has been tested in a series of clinical studies investigating type 2 diabetes, ulcerative colitis, and psoriasis (Krueger et al. 2015; Sands et al. 2016, Noh et al. 2017). Further clinical experiments are needed to determine whether these drugs have direct protective effects on vessels. Given that SIRT1 is an NAD^+-dependent enzyme, supplementing NAD is another method of activating SIRT1. The NAD level declines in ECs during aging, leading to a reduction in SIRT1 activity and endothelial dysfunction (Li et al. 2017; Das et al. 2018). NAD precursors such as nicotinamide mononucleotide and nicotinamide riboside have been shown to stimulate SIRT1 activity and delay vascular aging (Gomes et al. 2013).

In addition to drugs and small molecules, caloric restriction (CR) represents the only nongenetic regime that can prolong healthspan, and its beneficial effects on vascular aging have been demonstrated in many animal models. For example, CR has anti-atherogenic effects in *ApoE*$^{-/-}$ mice by reducing oxidative stress (Guo et al. 2002). Furthermore, CR can delay vascular inflammation by decreasing systemic inflammation and enhancing anti-inflammatory functions (Kalani et al. 2006). Our laboratory has shown that CR markedly reduces AAA formation and attenuates aortic elastin degradation in *ApoE*$^{-/-}$ mice by increasing the expression of SIRT1

(Liu et al. 2016). Furthermore, the metabolic reprogramming of the entire body during the CR process may reduce the risk factors of vascular aging; however, whether CR directly reprograms vascular metabolism requires further research.

Because it is inappropriate for patients to undergo long-term CR, future CR-related studies may benefit from the use of a CR mimetic (Redman et al. 2018). Many types of CR mimetics influence the glycolytic process and lipid and adipokine regulation, among others (Ingram et al. 2006). However, a single CR mimic may not be able to fully reflect the effects of CR; therefore, broader and deeper explorations of the mechanism of CR or combining multiple CR mimics may achieve better effects in preventing vascular aging.

4.7 Conclusion and Perspective

Vascular aging and related diseases are complex pathophysiological processes influenced by both genetic and epigenetic regulation. As summarized in this review, epigenetic factors influence vascular aging and related diseases through cell senescence, vascular dyshomeostasis, and vascular remodeling (Fig. 4.2), which fills the

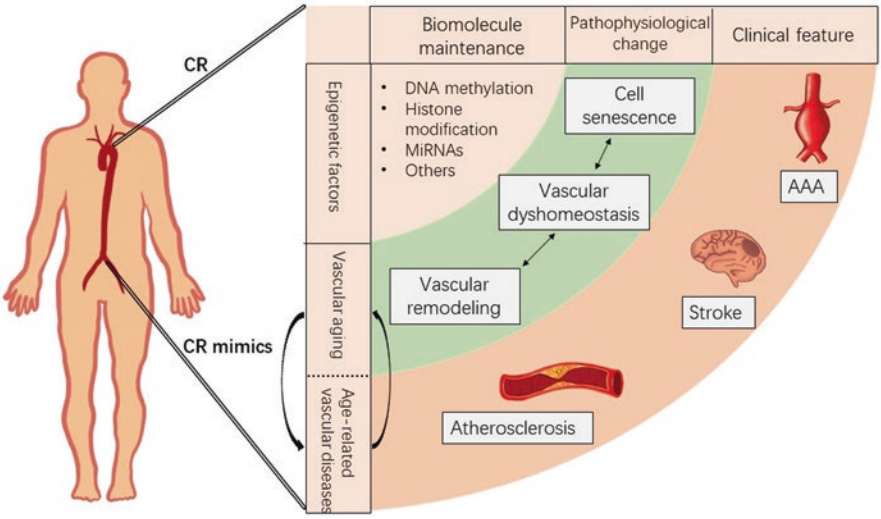

Fig. 4.2 Epigenetic regulation and vascular aging. Common epigenetic regulation mechanisms, such as DNA methylation, histone modification, and RNA-based gene regulation, all take part in the process of vascular aging. They influence the three characteristics of vascular aging, including cell senescence, vascular dyshomeostasis, and vascular remodeling. The arrows show the relationships among different layers. Moreover, both vascular aging and age-related vascular diseases, especially the AAA, stroke, and atherosclerosis, occur simultaneously and form a vicious circle. And because of the similarity and simultaneity, they may share the same epigenetic changes. Caloric restriction (CR) and small molecules that mimic the effects of caloric restriction can slow or even abolish some of the consequences of vascular aging by epigenetic regulation

gaps in disease pathologies that cannot be explained by human genetic and genomic research (Feinberg 2010; Feinberg et al. 2010; Schiano et al. 2015).

However, the current understanding of epigenetic mechanisms in vascular aging and age-related vascular diseases is only the tip of the iceberg. With the development of advanced bioinformatics tools and epigenetic methodologies, additional epigenetic factors will be identified. For example, the modification of RNA methylation is an emerging research field that has drawn increasing attention (Liu and Jia 2014; Flores et al. 2017). In the future, better methods, such as single-cell or low-cell-number sequencing methods (Nawy 2014; Shankaranarayanan et al. 2011), can be used to identify different epigenetic regulation patterns among senescent and normal cells. Importantly, the exact role of senescent cells that contributes to aging (we designated it as "senescaging") in the vascular system deserves further investigation; whether the epigenetic regulation of senescaging is independent of vascular dyshomeostasis and vascular remodeling remains unknown, and many studies must be performed before we achieve a deeper understanding. Therefore, a better understanding of the exact role of epigenetic regulation in vascular aging with advanced epigenetic-related methods may delay vascular aging. Furthermore, therapeutics targeting senescent cells may delay or reduce vascular aging and protect age-related vascular diseases.

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ApoE and Neurodegenerative Diseases in Aging

5

Yuemiao Yin and Zhao Wang

Abstract

Age and apolipoprotein E (ApoE) are the mightiest risk factors for dementia and cardiovascular diseases, but the underlying mechanisms remain unclear. In human, ApoE has three isoforms, ApoE2, ApoE3, and ApoE4, which are expressed by the polymorphic alleles: *ε2*, *ε3*, and *ε4*. Among the three polymorphic alleles, *apoE ε4* is the most risk gene. ApoE is the main ligand for the low-density lipoprotein (LDL) receptor and the LDL receptor-related protein (LRP), functioning as the component of plasma lipoproteins in the transportation of lipids. Physiologically, ApoE is a multifunctional protein with central roles in lipid metabolism; it transports lipids, including cholesterol, through the cerebrospinal fluid (CSF) and plasma. ApoE expression regulation and *apoE* gene polymorphism have an important connection with neurological or neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), ischemic stroke, and other diseases.

Keywords

Apolipoprotein E · Aging · Alzheimer's disease · Neurodegenerative diseases

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5.1 Introduction

Aging is a complex of biological long-lasting processes that result being unavoidable. Aging and diseases are closely related as aging is the largest risk factor for multiple chronic diseases. Increasing evidence suggests a certain degree of heritability of lifespan. Recently, genome-wide association studies (GWAS) candidate gene studies (CGAS) have identified variation in two genes (Fortney et al. 2015; Johnson et al. 2015), fork head box O3 (FOXO3) and apolipoprotein E (ApoE), to be consistently associated with human longevity, while some other genes have inconsistence (Blanche et al. 2001; Deelen et al. 2011; Schachter et al. 1994; Zhang et al. 1998). Furthermore, ApoE, which is involved in lipoprotein metabolism, is the only age-related gene confirmed in human (Bao et al. 2014; Fortney et al. 2015).

ApoE is a 34 kDa lipid-binding protein which was first discovered by Shore in 1973 in very-low-density lipoprotein (VLDL) (Shore and Shore 1974). It is mainly distributed in VLDL, chylomicron (CM), and their wreckage. ApoE plays an important role in lipoprotein metabolism. It not only can bind to LDL receptor but also bind to the hepatic cell membrane chylomicrons (CM), VLDL debris, and some HDL (which contains ApoE) receptors. The function of ApoE is transportation of triglycerides and cholesterol in multiple tissues (Bu 2009; Leduc et al. 2010; Puglielli et al. 2003; Wang et al. 2006).

Based on the pivotal role of ApoE protein in lipoprotein metabolism in the brain and in the periphery, its expression regulation and expression types have an important connection with Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS), vascular dementia (VD), cardiovascular diseases (CVD), type 2 diabetes mellitus (T2DM), and other diseases (Fig. 5.1).

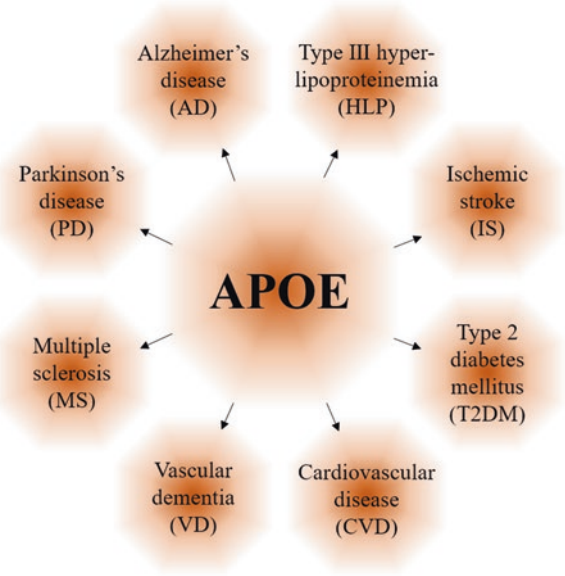


Fig. 5.1 Diseases associated with ApoE

5.1.1 The Structure and Physical Functions of ApoE

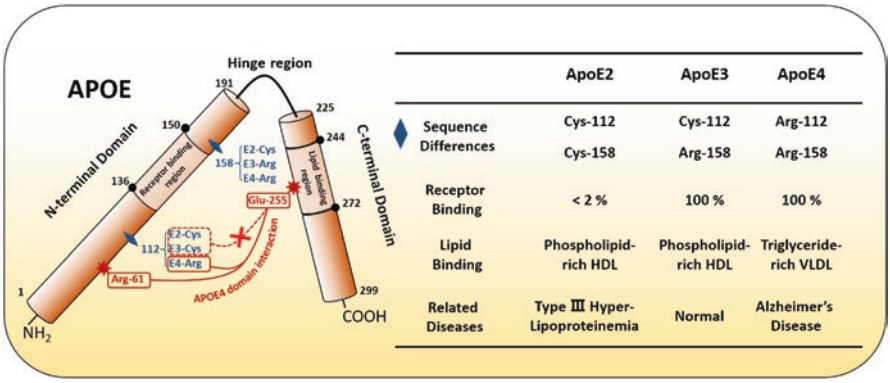
The molecular weight of ApoE is 34 kDa, consisting of 299 amino acid residues, rich in arginine with a single glycosylation site at threonine-194 (Lee et al. 2010; Rall et al. 1982; Weisgraber 1994). The secondary structure of ApoE was constituted of α-helix, β-turn β-sheet, and a "hinge region" which divides ApoE into two independent domains: the N-terminal domain (amino acids 1–191), two thirds of ApoE, contains the lipoprotein receptor-binding region (amino acids 136–150), and the C-terminal domain (amino acids 225–299) contains the lipid-binding region (amino acids 244–272) (Rasmussen 2016; Weisgraber 1994). X-ray crystallography solved the tertiary structure of the N-terminal domains of ApoE which consists of four helices arranged in antiparallel fashion (Weisgraber 1994), the lipoprotein receptor-binding region (amino acids 136–150) is in the fourth helix.

In contrast to other mammals, humans present three isoforms of ApoE, named ApoE2, ApoE3, and ApoE4, which are expressed by the polymorphic alleles: ε2, ε3, and ε4 (Tudorache et al. 2017). The substitution of Arg and Cys, two amino acid residues at positions 112 and 158 of the ApoE amino acid sequence, determines the type of isoforms: ApoE4 is Arg at both positions; ApoE2 is Cys; Cys at position 112 and Arg at position 158 are ApoE3 subtypes. The isoforms of ApoE display preferences for specific classes of lipoproteins, and ApoE4 prefers large, triglyceride-rich VLDL particles, whereas ApoE3 and ApoE2 associate preferentially with the small, phospholipid-rich HDL (Huang and Mahley 2014). The strange thing is the residues that differ the ApoE isoforms are in the N-terminal (E4, arginine 112; E3 and ApoE2, cysteine 112). However, the lipid-binding region is in the C-terminal (amino acids 244–272). This suggests that there may be a domain interaction between the N- and C-terminal domains in ApoE4; arginine 112 may orient the side chain of arginine 61 into the aqueous environment and then interact with glutamic acid 255, which determines the preference of ApoE4 for VLDL and of ApoE3 and ApoE2 for HDL (Huang and Mahley 2014) (Fig. 5.2).

In the 1970s, scientists found that ApoE is a component of a key modulator of lipoprotein, plasma lipoprotein, and cholesterol concentrations. Up to 75% of ApoE in plasma is synthesized by hepatic parenchymal cells (Mahley 1988); however, there are other organs and tissues producing a large amount of ApoE, most notably the brain, as well as the spleen, kidney, macrophages, and adipocytes (Ang et al. 2008; Getz and Reardon 2009; Williams et al. 1985). Physically, ApoE acts as cholesterol transporter, the key regulator to redistribute cholesterol within cells and to mobilize cholesterol between cells. These functions of ApoE transport cholesterol are essential for keeping myelin and neuronal membranes maintain both in the central and peripheral nervous systems (Leduc et al. 2010).

5.1.2 The Polymorphism of *apoE* Gene

The human *apoE* gene, 3.6 kb long, is located on the long arm of chromosome 19 and consists of four exons (Weisgraber 1994). Utermann first observed the



	ApoE2	ApoE3	ApoE4
Sequence Differences	Cys-112 Cys-158	Cys-112 Arg-158	Arg-112 Arg-158
Receptor Binding	< 2 %	100 %	100 %
Lipid Binding	Phospholipid-rich HDL	Phospholipid-rich HDL	Triglyceride-rich VLDL
Related Diseases	Type Ⅲ Hyper-Lipoproteinemia	Normal	Alzheimer's Disease

Fig. 5.2 Schematic illustration of structures of ApoE isoforms and its functional regions. The structure of ApoE constituted of two independent domains: the N-terminal domain (amino acids 1–191) contains the lipoprotein receptor-binding region (amino acids 136–150), and the C-terminal domain (amino acids 225–299) contains the lipid-binding region (amino acids 244–272). In ApoE4, two amino at position 112 and 158 differs the type of ApoE. The two arginines at position 112 and 158 in the N-terminal domain form a domain interaction with glutamic at position 255, which may determine the prior choice of ApoE4 for VLDL

polymorphism of *apoE* in 1975. Subsequent confirmation of the cDNA sequence directly tested revealed that there are three isoforms of *apoE* gene: *ε2*, *ε3*, and *ε4*. Some people only contain one major subtype, which is homozygous; some people can contain two main subtypes, namely, heterozygotes. Thus, there are six different phenotypes in the population, and all were readily detectable in human subjects: three homozygous phenotypes (*ε*4/4, *ε*3/3, and *ε*2/2) and three heterozygous phenotypes (*ε*4/3, *ε*3/2, and *ε*4/2) (Utermann et al. 1978, 1979a, b; Utermann and Beisiegel 1979).

In natural populations, *apoE ε3* allele is the most common (77.9%), *ε2* allele the least common (8.4%), and *ε4* in the medium (13.7%) (Farrer et al. 1997). The gene frequencies of ApoE in Chinese population are 0.88, 0.05, and 0.06. At the same time, ApoE is also involved in the normal growth of the nervous system and repair process after injury; the nervous system has a wide range of physiological and pathological effects. Because *ε3* appears to have the highest frequency, it is considered "wild type," *apoE* 2 and *apoE* 4 are due to its mutation, variant receptor binding than "wild type" decreased, ApoE2 receptor-binding activity was reduced to 1% of the activity of ApoE3, and the decrease in ApoE2 receptor binding is closely related to inherited lipid disorders. The mutation of the gene *apoE* is involved in the pathogenesis of some of the primary cases of Alzheimer's disease. *apoE* 2 has protective effects on vascular integrity; *apoE* 3 is moderate, while *apoE* 4 causes a fivefold increase in vascular inflammatory factor CypA, making blood vessels brittle, and also increases the risk of getting Alzheimer's disease. However, people who have this genetic variant do not necessarily have Alzheimer's disease. On the contrary, those who do not have this genetic variant are equally likely to have Alzheimer's

disease. So apart from genes, scientists suspect there must be more environmental factors that contribute to the development of Alzheimer's disease.

5.2 ApoE and Alzheimer's Disease

5.2.1 Alzheimer's Disease

Alzheimer's disease (AD) is a common neurodegenerative disease among elder people which cannot be cured. The most adverse effects are cognitive decline and memory loss. Approximately 13% of elder people over the age of 65 and 45% over the age of 85 presently are affected by AD (Assoc, A 2012). There are at least 30 million AD patients around the world, and it will reach 131 million in 2050 (ADI 2016; Hung et al. 2016). Due to an increasing elder population, AD becomes one of the greatest health issues of this century (Hickman et al. 2016) and is definitely the sixth leading cause of death in the USA (Assoc, A 2015). In 2016, the total health-care costs including long-term care and hospice services, for people aged over 65 years with dementia, are estimated to be $236 billion, and this number will be doubled in 2030 (Association 2016) .

Compared with the healthy brain, AD patients' brain has severe shrinkage, especially in the hippocampus. Histopathology shows that extracellular senile plaques and intracellular neurofibrillary tangles are two hallmarks of AD pathology (Kanekiyo et al. 2014). Senile plaque involves amyloid-β peptide's (Aβ) abnormal accumulation and aggregation between the neurons and later forms depositions in the gray matter of the brain, mainly in the hippocampus (which involves in new memory formation) and neocortex (Luo et al. 2017), while neurofibrillary tangles are associated with tau hyper-phosphorylation. However, due to the complex genetic, epigenetic, and environmental factors that may influence the development of AD, the mechanisms of AD have not been fully studied. Strong evidence suggest that human *apoE* gene is the strongest genetic risk factor for LOAD known so far. Among the three isoforms, ε2, ε3, and ε4, the risk ranking of suffering AD is ε4 > ε3 > ε2. ApoE ε4 increases AD risk about ~3 (single allele)- and 15-fold (double allele), respectively, while ApoE ε2 can decrease the possibility of AD (Kim and Tsai 2009; Koffie et al. 2012; Saunders et al. 1993).

5.2.2 Role of ApoE in Alzheimer' Disease

Amyloid cascade hypothesis (ACH) has been proposed in 1992. The hypothesis mainly stands for the point of view that the deposition of Aβ, which is the major component of the senile plaques formed in AD patients' brains, is the upstream initiation factor of AD pathology. Aβ deposition finally induces neurofibrillary tangles, neuronal loss, cell death, and dementia (Hardy and Higgins 1992). Currently, a new modified ACH has been proposed by Karran E (Karran and De Strooper 2016). The modified ACH suggests that tau dysfunction may run in parallel with the

deposition of Aβ, but the key event in AD pathology is still Aβ deposition (Ricciarelli and Fedele 2017). However, others proposed different views: Moir suggests Aβ plaque may not be responsible for AD occurrence; on the contrary, Aβ wraps harmful pathogens to prevent them from infecting the brain, it is like the body's immune response, rather than the killer (Kumar et al. 2016).

Abundance of evidences has suggested *apoE* gene is the strongest genetic risk factor for LOAD, but the role ApoE plays in AD hasn't been fully explained. ApoE is primarily produced by the liver and macrophages in peripheral tissues, while it is produced by astrocyte or glia cells in the brain (Liu et al. 2013), both in humans and animals, and serves as a cholesterol carrier and mediates the uptake of lipoprotein particles (Hirsch-Reinshagen et al. 2009). ApoE mediates cholesterol metabolism in an isoform-dependent manner (Kanekiyo et al. 2014). It was demonstrated that ApoE4 has preference to very-low-density lipoproteins (VLDL), while ApoE3 and ApoE2 have a preference for small high-density lipoproteins (HDLs) due to their different structure sequence (Huang and Mahley 2014; Mahoney-Sanchez et al. 2016).

In human, compared to ε2 and ε3, the presence of ε4 is associated with increased risk for suffering both EOAD and LOAD, especially LOAD. Studies reveal that there is a clear relevance between *apoE ε4* and the neural disorder pathology in AD (Mahley et al. 2006). Genetic studies demonstrate that, among persons who inherit double ε4 alleles, the risk of suffering from AD by 85 years of age is 50–90%, and among persons with one ε4 allele is 45% (Xu et al. 2006).

Although there is a clear correlation between *apoE* ε4 gene and the elevated risk of AD, the mechanism for effect of ApoE in AD is complex and multi-angled. ApoE is associated with many aspects of AD (Arbor et al. 2016), both in Aβ-dependent and Aβ-independent ways, including Aβ metabolism, Aβ plaque formation, cytoskeletal structure and mitochondrial function impairment, synaptic plasticity loss, and blood-brain barrier (BBB) integrity impairment (Fig. 5.3).

5.2.2.1 APOE4 Affects AD in Aβ-Dependent Way

Aβ production and clearance disturbance may play a central role in AD pathogenesis. Obviously, ApoE relates to Aβ metabolism in AD in isoform-dependent manner. There are evidences to indicate that levels of soluble Aβ are increased with ApoE4, providing a potential mechanism of ApoE4-induced AD risk (Tai et al. 2014). However, the pathways by which ApoE4 may increase Aβ levels are unclear. Based on the existing evidences, ApoE may affect Aβ by the pathways of forming complexes, interfering Aβ clearance, altering Aβ degradation enzyme, and facilitating Aβ plaque formation.

Some research suggest that ApoE can directly interact with Aβ. Histological analyses of AD patients' brains show that ApoE is co-deposited with Aβ in amyloid plaques (Namba et al. 1991). Epitope mapping demonstrates that residues 144–148 in the ApoE N-terminal region can interact with residues 13–17 in Aβ, forming the ApoE/Aβ complexes (Cho et al. 2001) which interfere Aβ uptake ways. Purified ApoE4 can bind to Aβ with a higher affinity than ApoE3 and E2 (Ladu et al. 1994). Researches have shown that ApoE increases the level of Aβ oligomers in an isoform-dependent manner (E4 > E3 >E2) (Hashimoto et al. 2012; Youmans et al. 2012).

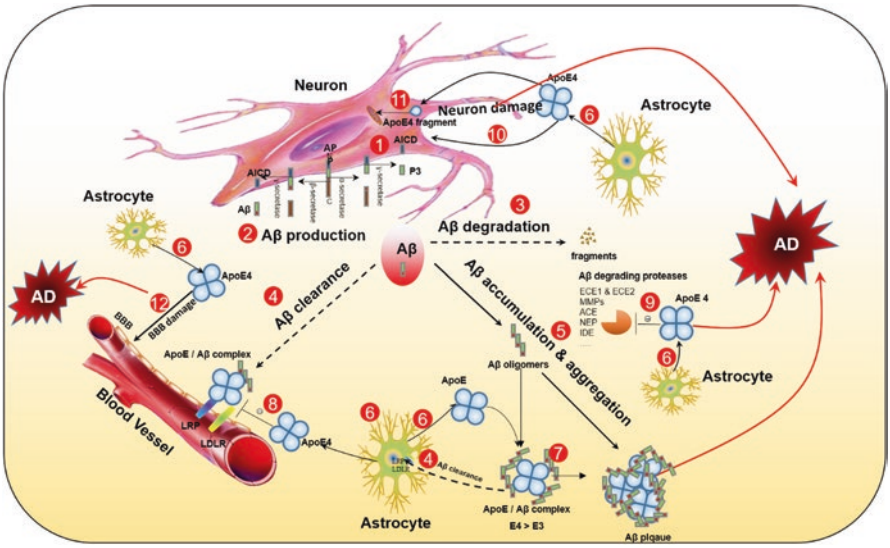


Fig. 5.3 Aβ metabolism in the brain and Aβ-dependent/Aβ-independent effects of ApoE on Alzheimer's disease. (*1*) Non-amyloid metabolic pathway of amyloid precursor protein (APP); (*2*) amyloid metabolic pathway of APP produces amyloid-β peptide (Aβ); (*3*) Aβ is degraded by insulin-degrading enzyme (IDE) and neprilysin (NEP). (*4*) The major Aβ clearance pathways include receptor (LRP/LDLR)-mediated uptake into astrocyte/microglia cell or through the blood-brain barrier (BBB). (*5*) Extreme Aβ accumulation and aggregation can promote Aβ oligomers and Aβ plaque formation which leads to AD. (*6*) Apolipoprotein E (ApoE) is mainly produced by astrocyte in the brain. (*7–9*) Aβ-dependent effects of ApoE on AD: (*7*) ApoE directly interacts with Aβ and interferes Aβ clearance. (*8*) ApoE4 competes with Aβ for the same receptor LRP and LDLR, which interferences the cellular uptake pathways of Aβ. (*9*) ApoE4 inhibits Aβ-degrading enzymes to downregulate Aβ degradation. (*10–12*) Aβ-independent effects of ApoE on AD: (*10*) ApoE4 can directly damage neuron and leads to AD; (11) C-terminal of ApoE4 enters cytosol causing mitochondrion dysfunction; (12) ApoE4 impairs blood-brain barrier (BBB) integrity

Moreover, blocking the ApoE/Aβ interaction can relieve Aβ-related pathology including brain Aβ accumulation, co-accumulation of ApoE within Aβ plaques, and neurodegeneration in both APP/E2 and APP/E4 mice (Pankiewicz et al. 2014).

ApoE can also modulate Aβ clearance way as a competitor. All three isoforms of ApoE can bind to the receptors and transporters such as low-density lipoprotein (LDL) receptor-related protein (LRP) in astrocytes that supposed to bind Aβ, which form a competition of Aβ cellular uptake pathway (Verghese et al. 2013). Interestingly, compared to ApoE4, ApoE2 and ApoE3 cleared more Aβ in transgenic mice (Dodart et al. 2005; Hudry et al. 2013).

Our previous research has shown that ApoE can also regulate Aβ metabolism by affecting its degrading enzyme IDE extracellularly. ApoE4 significantly downregulates the expression of IDE, while ApoE3 could rescue these effects in ApoE knockout mice (Du et al. 2009a). Keeney' research also demonstrated that ApoE4 mice exhibited downregulated peroxisome proliferator-activated receptor (PPARγ) levels and IDE expression (Keeney et al. 2015). In another research of our lab, we suggest

that PPARγ could transcriptionally activate IDE gene expression (Du et al. 2009b). These results indicate that ApoE4 may decrease IDE expression by inhibiting PPARγ.

Furthermore, some studies suggest that ApoE isoforms on AD pathogenesis are through plaque formation. Holtzman's researches provide evidences that APPsw mice carried two *apoE* (+/+) and one (+/−) presented more Aβ plaques than no copies (−/−) of normal mice *apoE* gene (Holtzman et al. 2000b). In addition, they further demonstrate that these effects of ApoE are isoform specific (E4>E3) (Holtzman et al. 2000a).

5.2.2.2 APOE4 Affects AD in Aβ-Independent Way

In addition, both in vivo and in vitro studies also suggest ApoE may affect AD in Aβ-independent ways in parallel with Aβ-independent ways, including synaptic plasticity, BBB integrity, cytoskeletal structure and mitochondrial function impairment, synaptic plasticity loss, and blood-brain barrier (BBB) integrity impairment.

ApoE4 causes neuronal and behavioral deficits in the absence of Aβ accumulation in transgenic mice. Transgenic mice expressing human ApoE3 or ApoE4 and lacking endogenous mouse ApoE have been established (Buttini et al. 1999; Raber et al. 1998). Among all these models, Aβ levels do not accumulate; however, ApoE4 mice show deficits in vertical exploratory behavior and impairment of spatial learning and memory, while ApoE3 mice and wild-type mice show no significant change, and these impairments of learning and memory are gender specific (female>male) (Buttini et al. 1999; Raber et al. 1998).

ApoE impairs synaptic plasticity in an isoform-dependent manner. As compared to ApoE3, ApoE4 decreases dendritic spine density in transgenic and gene-targeted mice (Jain et al. 2013). ApoE3 promotes neurite outgrowth and increases neuronal sprouting (Kim et al. 2014). However, the effect of ApoE4 on synaptic plasticity is inconsistent. A study reported that ApoE4 had prejudicial effects on neurite outgrowth (Teter et al. 2002), while another study suggested ApoE4 even had stimulating effects in the absence of Aβ (Puttfarcken et al. 1997).

Moreover, it has also been demonstrated that the C-terminal fragments of ApoE4 can enter the cytosol and cause neurotoxicity by disrupting the cytoskeleton (Huang et al. 2001). ApoE4 fragment also target the neuron mitochondrion, leading to mitochondrial dysfunction. Brodbeck's research later demonstrate that ApoE decreases mitochondrial mobility in an isoform-specific manner (E4 fragment > E4 > E3) (Brodbeck et al. 2011; Chang et al. 2005).

On the other hand, ApoE also exhibits isoform-specific effects on BBB integrity in mouse models (Bell et al. 2012). In both human *apoE* gene knock-in and glial fibrillary acidic protein promoter transgenic mice, ApoE4 expression increases the susceptibility of BBB to injury in the absence of Aβ. It has been reported that pericytes express ApoE (Xu et al. 2006), which might lead to BBB damage in the context of ApoE4.

5.3 ApoE and Other Neurodegenerative Diseases

Although the linkage is not as strong as with AD, ApoE also associates with progression in other neurological or neurodegenerative diseases, including Parkinson's disease (PD), vascular dementia (VD), multiple sclerosis (MS), traumatic brain injury (TBI), ischemic stroke (IS), etc.

5.3.1 ApoE and Parkinson's Disease

Though PD has some clinical and neuropathological features that are similar with AD, there are still lots of inconsistent features. Compared to AD, PD progresses slowly in most people, affecting less of the population older than 65 years of age (PD 2% vs AD 13%) (Hughes et al. 1993). Until now, the association between ApoE and PD is still controversial. Hardy's research notice a strong association between the *apoE* ε4 allele and AD but no association between the *apoE* ε4 allele and PD (Hardy et al. 1994). Also, *apoE* ε4 does not aggravate AD lesion in patient with PD (Egensperger et al. 1996). Li and Pulkes's researches, however, demonstrate the association between ApoE and PD in CNS (Li et al. 2004; Pulkes et al. 2011). Another research demonstrate *apoE* ε2 is associated with higher risk of PD development (Huang et al. 2004). So far, the role of ApoE in PD remains a lot of inconclusive.

5.3.2 ApoE and Vascular Dementia

VD is a severe cognitive impairment caused by brain damage from impaired blood hypoperfusion in the brain and usually happens after suffering ischemic stroke, hemorrhagic stroke, and cerebrovascular diseases (Roman 2004). VD is one of the second common causes of dementia after Alzheimer's disease, causing around 15% of cases (O'Brien and Thomas 2015). Clinically, VD presents pathological features such as the amyloid plaques, neurofibrillary tangles, and white matter lesions, same as AD (Kalaria 2003). There are many risk factors of VD, including hypertension, ischemic stroke, hemorrhagic stroke, atherosclerosis, and other metabolic disorders; in addition to the above, ApoE is also considered as an important risk factor for VD, but the conclusions are conflicting. Some studies demonstrate there is a positive association between *apoE* ε4 allele and increased risk of VD (Baum et al. 2006; Chuang et al. 2010; Yin et al. 2012); on the contrary, Kawamata's research find no obvious association between *apoE* ε4 allele and VD in Japanese (Kawamata et al. 1994).

5.3.3 ApoE and Multiple Sclerosis

MS is the most common demyelinating disease of the central nervous system. MS usually occurs between the ages of 20 and 50 and more common in women than men. The lesions are characterized by multiple lesions, remissions, and recurrences in the optic nerve, spinal cord, and brain stem (Zephir 2018). So far, some researches demonstrate a negative association between *apoE* ε4 allele or ε2 allele and MS (Carmona et al. 2011; Ghaffar et al. 2010; Ramagopalan et al. 2007; Xuan et al. 2011; Zwemmer et al. 2004) or MS patients' cognitive impairment (Portaccio et al. 2009), while some researches indicate *apoE* ε4 carriers with MS have worsening progression of cognitive deficits than noncarriers (Oliveri et al. 1999; Shi et al. 2011). In summary, the possible relationship between the *apoE* ε4 allele and cognitive dysfunction in MS patients is small and on balance suggests a link.

5.3.4 ApoE and Ischemic Stroke

Stroke is a medical condition in which poor blood flow to the brain results in cell death. There are two main types of stroke: ischemic stroke (IS), due to lack of blood flow, and hemorrhagic stroke (HS), due to bleeding. Due to the association between *apoE* ε4 allele and increased levels of LDL and cholesterol, ApoE may have an impact on IS occurrence; several meta-analyses report a significant association between IS and the *apoE* ε4 allele.(Das et al. 2016; McCarron et al. 1999; Wang et al. 2006; Xu et al. 2016). It has been demonstrated that *apoE* ε4 carrier patients have significantly greater risk of IS occurrence (Treger et al. 2003). Also, *apoE* ε4 allele is related to increasing carotid intima-media thickness, which is associated with IS (Paternoster et al. 2008). IS is a result of combination interactions between environmental and various genetic factors: *mthfr*, *apoE*, *pon1*, *pde4d*, etc. (Wei et al. 2017), and influence of each gene is not as strong as in AD but is expected to be modest. The influence of genetic factors may be obscured by the acquired risk factors in IS. However, *apoE* gene seems to be a strong candidate for studying the interplay between genetic and acquired risk factors (Van Giau et al. 2015).

5.4 Conclusion

ApoE is a kind of apolipoprotein closely related to the nervous system. Its genetic polymorphism is not only closely related to lipid metabolism but also closely related to various neurological or neurodegenerative diseases and cardiovascular diseases, such as AD, PD, VD, MS, and IS. This review highlighted the association between ApoE and neurodegenerative diseases. The association between ApoE (especially ApoE4) and AD is strong and has been known for decades; several theories have been proposed how ApoE plays its roles, both in Aβ-dependent and Aβ-independent pathways. On the contrary, the linkage between ApoE and other neurological or neurodegenerative diseases is not as strong as AD, the effect of ApoE expression

and ApoE polymorphism is also controversial, and this may be explained by the complex of the influences of genetic factors and environment factors (acquired factors). In summary, the association between ApoE and the risk of pathogenesis is still not clear, but ApoE is a definite essential factor for diagnosis, risk assessment, prevention, and treatment of disease in humans.

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Brain Aging: Hsp90 and Neurodegenerative Diseases

6

Kun Wang, Yu Shang, and Fei Dou

Abstract

The brain is the most complex organ in the human body and the main component of the central nervous system. Because it lacks the ability of regeneration, age is a major risk factor for most common neurodegenerative diseases, which caused an irreversible cognitive impairment. It has been shown that the function of molecular chaperones, majorly heat shock proteins, was compromised and then causes the imbalance of protein homeostasis inside the cell, which is the most influential reason of brain aging. Here, in this review, we discuss the mechanisms underneath the impairment of heat shock protein function during brain aging, including transcriptional regulation, posttranslational modification, and communication across cells and organs.

Keywords

Molecular chaperone · Heat shock protein · Proteostasis · Brain aging

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6.1 Introduction

Aging is always an intriguing topic for everyone. It is the primary risk for major human pathologies, including cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases. Recently, after thoroughly reviewing the current understanding of molecular mechanism of aging, nine hallmarks of aging were proposed (López-Otín et al. 2013). Those hallmarks are genomic instability, telomere attrition, epigenetic alteration, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication.

In the nine hallmarks, the first four considered the primary causes of damage. In multicellular organisms, the primary cause of aging may not be the same for different organs. Tissues such as the blood, skin, bone, and gut have high turnover rates, with "old" tissue being constantly replaced throughout our lifetime; genomic instability and telomere attrition may be the major causes of aging. Conversely, in brains, hearts, and muscles that have lost the ability of spontaneous regeneration, highly differentiated cells have entered the G0 phase of the cell cycle, and no more chromosomes are replicated, so the stability of the genome and the length of telomeres are not the main factors of these organs' aging. In postmitotic cells such as neurons, the loss of proteostasis may be a major cause of aging. Because in these tissues toxic substances cannot be diluted by cell division, misfolded or damaged proteins accumulate as they age.

In order to maintain protein stability in cells with limited proliferative potential, such as neurons, quality control system is very important in refolding or degrading misfolded proteins. Heat shock proteins are essential component of the intracellular protein quality control system. The other two important components of protein quality control system are ubiquitin-proteasome system and autophagy-lysosome system. The latter two systems control the protein degradation, while HSPs are involved in the whole lifespan of proteins from nascent protein folding to damaged protein degradation (Höhfeld et al. 2001). Once the balance between misfolded/damaged proteins and free HSPs becomes disturbed, it may cause aging-related diseases such as Alzheimer's disease and Parkinson's disease.

6.2 The Involvement of Heat Shock Proteins in Neurodegenerative Diseases

The involvement of heat shock proteins in several different neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease (PD), and Huntington's disease (HD), has been well studied.

On the one hand, accumulated evidences suggest the potential for therapeutic manipulation of chaperones in neurodegenerative diseases. Auluck et al. have found that chaperones can attenuate neurotoxicity in a *Drosophila* model of PD by influencing the conformation of a-synuclein (Auluck et al. 2002). It also has been demonstrated that in HD, a model of polyglutamate tract diseases, molecular chaperones

partition huntingtin aggregates from a cytotoxic, fibrillar form to an amorphous, non-cytotoxic form (Warrick et al. 1999; Carmichael et al. 2000; Muchowski et al. 2000; Sittler et al. 2001; Slavotinek and Biesecker 2001). Additionally, our own results suggest that molecular chaperones may suppress formation of neurofibrillary tangles by preventing tau aggregation and partitioning tau into a productive folding pathway and by accelerating degradation of aberrantly folded tau (Dou et al. 2003a, b, 2005, 2007; Luo et al. 2007).

At the same time, many studies have shown that neurodegenerative diseases accompany with abnormal expression, localization, and function of molecular chaperones. Uryu et al. detected PD brain tissue and found that there was a clear co-localization of Hsp90 and α-synuclein in Lewy bodies compared to other heat shock proteins. In PD mouse models, the authors also found similar phenomena. In the cell model overexpressing α-synuclein, the authors found that the expression of Hsp90 was significantly up-regulated (Uryu et al. 2006).

Yokota et al. analyzed the gene expression changes in the temporal and occipital lobe of AD patients using the PCR-select cDNA subtraction method and found that Hsp90 expression was significantly downregulated in brain tissue of AD patients compared with normal controls (Yokota et al. 2006). Gezen-Ak et al. collected the serum from patients of early-onset AD (EOAD), late-onset AD (LOAD), and mild cognitively impaired (MCI) and age-matched healthy controls and then detected the protein level of brain-derived neurotrophic factor (BDNF), complement factor H (CFH), tumor necrosis factor-α (TNFα), interleukin-10 (IL-10), and Hsp90 by ELISA. It was found that the Hsp90 levels in the serum of EOAD, LOAD, and MCI patients significantly decreased compared with the normal controls (Gezen-Ak et al. 2013).

Based on the existing research results, we speculate that during the aging of the brain, the expression and function of the chaperones are impaired due to a variety of reasons. The first reason is the imbalance of proteome and chaperome; second, the induction of heat shock protein gene expression is repressed; third, posttranslational modifications cause the function damage of heat shock proteins; and fourth is the proteotoxic transfer across cells and organs. In this review, we will discuss the molecular mechanism underneath this impairment in neurons and the consequences of it (Fig. 6.1).

6.3 The Imbalance of Proteome and Chaperome During Brain Aging

Many evidences confirm the relevance between proteome imbalance and aging. Recently, a research group has studied the expression profile of more than 5000 proteins in the entire life cycle of *C. elegans*. The results show that one-third of the protein's abundance changes at least two times during aging, leading to severe proteome imbalance. Proteome imbalance can alter protein stoichiometry and lead to proteostasis stress, accompanied by extensive protein aggregation. If these aggregates can recruit enough chaperones to bind, then the individual's lifespan can be

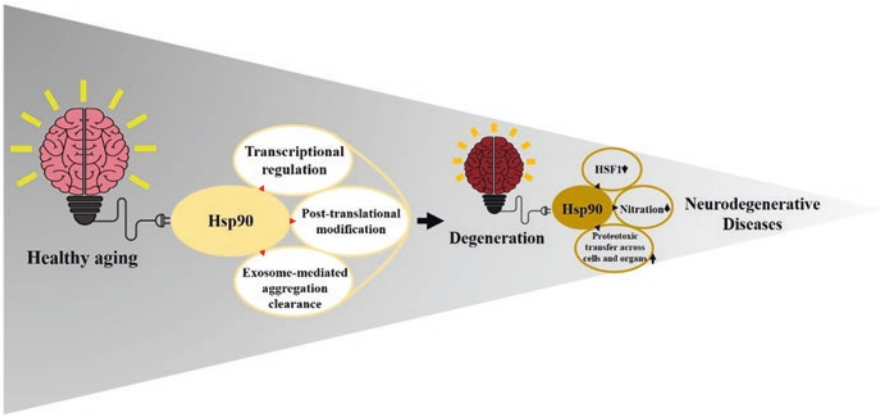

Fig. 6.1 Brain degeneration during aging caused by molecular chaperone Hsp90 disturbance. Molecular chaperone Hsp90 is induced during brain aging process and plays an important role in protein homeostasis maintenance inside the cell. Through rightful transcriptional regulation, post-transcriptional modification, and communication across cells and organs, Hsp90 insures brain aging in a healthy way. On the contrary, if Hsp90 is compromised, a series of biological process will be disturbed, such as heat shock response, protein modification changes, and proteotoxic transfer, and thus will cause brain degeneration together with neurodegenerative diseases

prolonged, suggesting that isolating abnormal proteins delays the decline of protein levels during aging (Walther et al. 2015). Similar future studies on the aging mammalian proteome should help to clarify the universality of the central role of changes in protein abundance in the loss of proteostasis with age.

In another study, Brehme et al. have examined the chaperome in *C. elegans* and human. They find that chaperome expression is dramatically affected in human brain aging. At the same time, chaperome dynamics are associated with aging and neurodegenerative diseases. By using the gene expression data from the superior frontal gyrus (SFG) of 48 brains from normal individuals of 20–99 years, aging correlation analysis of the human chaperome expressed in the SFG was performed. They identified 101 chaperone genes (31.8%) that are repressed and 62 (19.5%) genes that are induced during aging. Chaperome age expression revealed enrichment of certain functional families in induction and repression clusters. Next, they evaluated the effects of AD, HD, and PD on chaperome dynamics by examining the expression in patients' brain samples. When analyzing brain expression data sets from AD patients, 101 significantly repressed genes and 34 induced genes were identified compared to age-matched controls. In both aging and AD brains, chaperome repression is significantly enriched compared to overall gene repression in the genome. They also find that the chaperome genes differentially regulated in AD overlapped with the aging-regulated chaperome genes (Brehme et al. 2014). In summary, on the one hand, proteome imbalance occurs during the aging process of the brain, leading to the formation of aggregates by many highly abundant proteins; at the same time, the expression of many molecular chaperones within cells is repressed. The superposition of the two factors further exacerbates the imbalance of the proteome and accelerates the aging of the brain or the occurrence of neurodegenerative diseases.

The main players in proteostasis maintenance are chaperones and two proteolytic systems, the ubiquitin-proteasome and the lysosome-autophagy systems. For reasons still unknown, aging has a negative effect on the cross talk between proteolytic pathways. Using a model of proteasome stress in rat hippocampus, Gavilan et al.'s study confirmed the functional cross talk between the ubiquitin-proteasome system and the autophagy-lysosomal pathway and also found that the cross talk between the two proteolytic systems weakened with aging. Under proteasome inhibition, both autophagy activation and resolution were efficiently induced in young but not in aged rats, leading to restoration of protein homeostasis only in young pyramidal neurons (Gavilán et al. 2015). In another study, by using a mouse model with liver-specific deficient chaperone-mediated autophagy (CMA), they confirmed the changes in protein homeostasis, due to decreased CMA activity in the organ with age. In addition, they also found that other proteolytic systems could compensate for the loss of CMA in young mice, which helps maintain protein homeostasis. However, these compensatory responses are not sufficient to prevent aging-related protein toxicity (Schneider et al. 2015).

6.4 The Decline of Heat Shock Response During Aging

The potential for heat shock response declines throughout the life cycle, and the weakening of this response contributes to aging by allowing the appearance of protein-cluster diseases, reduced cell viability, and reduced longevity. It has recently been suggested that an increase in protein damage during aging may be exacerbated by a decrease in heat shock response, a decrease in the level of heat shock protein (HSPs), and a resulting loss of protein quality control. This pathway appears to be regulated in mammalian cells by several routes.

Increased damaged protein activates HSF and results in a constitutively elevated level of heat shock protein commonly observed in older organisms. However, long-term high levels of molecular chaperones, particularly Hsp90, inhibit the transcriptional activity of HSF, making the ability of older organisms to respond to stress stimuli significantly diminished. At the same time, the activation and binding of HSF and heat shock elements in aged animals and cells also decreased.

Kregel et al. observed the aging-dependent decline of the heat shock response both in cell culture and animal (Kregel 2002). Fawcett et al. also reported that the stress-induced DNA-binding activity of HSF1 and heat shock protein levels are markedly reduced in 21–26-month-old rats when compared with adult 5–6-month-old rats (Fawcett et al. 1994; Locke and Tanguay 1996). This decline does not correlate with a decline in HSF1 protein, as the amount of HSF1 appears to remain unchanged from young adult to aged rats. A similar age-dependent reduction in HSF1 DNA-binding activity can be seen when human lymphocytes and skin fibroblasts were isolated from young (20–40 years) or old (>70 years) donors and cultivated in vitro (Jurivich et al. 1997; Gutsmann-Conrad et al. 1998; Anckar and Sistonen 2011).

By contrast, Bonelli et al. reported that cellular senescence may unmask a proteasomal activity leading to the degradation of HSF1 under stress condition. Their

results show that, following serum deprivation of late passage human fibroblasts, the degradation of HSF1 accelerated. The author suggests that either heat shock unmasks a latent proteasome activity responsible for degradation of HSF1 or serum starvation induced posttranslational structural modifications of HSF1 in late passage cells, which would become a substrate for a proteasome at high temperature (Bonelli et al. 2001).

The discrepancy between these studies may be due to the different cell types used. In different cell types, aging may have different effects on proteostasis and chaperome homeostasis. Regardless, based on the above results, HSF1 activity declines during the aging of cells and organs; in some types of cells and organs, HSF1 also degrades rather than activates when the aging cells are under pressure challenge, which makes the situation worse and eventually causes cell death.

6.5 Posttranslational Modifications Cause the Function Damage of Heat Shock Proteins

Posttranslational modifications (PTMs) have been reported to regulate heat shock protein activity directly; these include phosphorylation, acetylation, SUMOylation, methylation, ubiquitylation, and S-nitrosylation and have been extensively reviewed in several papers (Mollapour and Neckers 2012; Zuehlke et al. 2015; Prodromou 2016). Here, we will focus on two kinds of PTMs that related to aging process.

The first modification is acetylation. Hyper-acetylated Hsp90 appears to have a lower affinity for ATP (Nimmanapalli et al. 2003). Acetylation of Hsp90 inhibits the formation of a functional Hsp90 complex by inhibiting ATP binding. The enzyme responsible for the acetylation of Hsp90 is not yet known, but the enzymes responsible for deacetylating Hsp90 are well studied. Deacetylation of Hsp90 appears to be regulated in mammalian cells by histone deacetylase 6 (HDAC6), which originally reported can bind to polyubiquitinated proteins and microtubules (Bali et al. 2005; Murphy et al. 2005; Boyault et al. 2007). Accordingly, HDAC6 and Hsp90 form a complex in vivo and that HDAC6 functions as an Hsp90 deacetylase to regulate its chaperone functions. HDAC6 would stimulate Hsp90 activity by catalyzing its deacetylation, enhancing ATP binding, and thereby promoting the assembly of functional Hsp90 chaperone complexes (Kovacs et al. 2005). Interestingly, HDAC6 is also involved in the regulation of HSF1 activation, which is required for HSF1 activation during inhibition of proteasome activity. Several studies have shown that HDAC6 is also involved in regulating HSF1 activity. When the proteasome activity in the cell is inhibited, the level of polyubiquitinated protein in the cell rises. HDAC6 can sense this change, induce the dissociation of the Hsp90 and HSF1 complexes, and then lead to the activation of HSF1 and transfer into the nucleus to initiate the heat shock response. Thus, HDAC6, in addition to its role in aggresome formation, participates in molecular chaperone synthesis and activity regulation (Boyault et al. 2007; Calderwood et al. 2009; Du et al. 2014; Pernet et al. 2014).

The second PTM of heat shock protein correlated with aging is tyrosine nitration. In the presence of reactive oxygen species (ROS), NO-derived reactive

nitrogen (RNS) such as peroxynitrite ion ($ONOO^-$) is an important part of the pathological injury. $ONOO^-$ can make tyrosine nitrification of protein tyrosine residues. Protein tyrosine nitration is an important posttranslational modification of proteins and is associated with neurodegenerative diseases, inflammation processes, and aging. Franco et al. show that nitration of a single tyrosine residue on a small proportion of 90-kDa heat shock protein (Hsp90) is sufficient to induce motor neuron death by the P2X7 receptor-dependent activation of the Fas pathway. Nitrotyrosine at position 33 or 56 stimulates a toxic gain of function that turns Hsp90 into a toxic protein (Franco et al. 2013). In a follow-up study, they further show that nitrated Hsp90 associates with mitochondria and regulates mitochondrial activity without inducing the release of cytochrome c. Her research team found that the nitrification of Hsp90 can limit the amount of oxygen that reaches the mitochondria of cells, thereby reducing their energy production. Therefore, such site-specific nitration of tyrosine 33 on Hsp90 confers a gain of function responsible for the regulation of mitochondrial activity, which is a trigger for killing cells in the nervous system of patients with degenerative diseases (Franco et al. 2015).

6.6 Proteotoxic Can Transfer Across Cells and Organs

Very little is known how one cell type or organ responds to protein misfolding signals from another compartment until recently (Van Oosten-Hawle et al. 2013; Takeuchi et al. 2015). The transfer of proteotoxic stress between organs may lead to aging-related pathologies and degenerative diseases. Unlike previous studies of proteostasis networks, these new studies have focused on the cross talk of different organs in the regulation of proteostasis networks under stress conditions. On contrast, previous studies have been limited to the proteostasis networks in the cytoplasm of specific organs. This comprehensive view of protein inhibition is important for further understanding of the basis and consequences of reduced protein inhibition in aging, and it has generated considerable interest as an unexploited therapeutic target for the treatment of age-related diseases.

It has been reported that HSPs, such as Hsp27, Hsp70, and Hsp90, are secreted from cells and that this process is significantly activated by heat stress and physical stress, including exercise (Hightower and Guidon Jr 1989; Walsh et al. 2001; Graner et al. 2007). However, the functional role of this secretion has remained unclear. Recently, Takeuchi et al. reported that each cell, in addition to maintaining its own protein homeostasis through molecular chaperones, can also maintain intracellular protein homeostasis with the help of other cells. By using polyglutamine disease in cell culture and *Drosophila* models, they provide direct evidence that proteostasis is indeed non-cell autonomously maintained in some cells by molecular chaperones expressed in other remote cells. Once misfolded proteins organize into oligomers or insoluble aggregates, the only options for their elimination from the cytosol are either by degradation in lysosomes through macroautophagy (MA) or expulsion outside the cell by means of small vesicles (exosomes). Exosomes have emerged as important organelles in cellular intercommunication (Lo Cicero et al. 2015).

Originating from the invagination of the endosomal membrane, these small vesicles trap samples of the cytosol that are then released to the extracellular environment upon the fusion of multivesicular endosomes with the plasma membrane. Exosomes can act as vehicles for the exchange of chaperones and maybe for other proteostasis effectors. Therefore, this exosome-mediated secretion and the chaperone's intercellular delivery are responsible for this non-cell-autonomous maintenance of proteostasis (Takeuchi et al. 2015).

Chaperones can be detected in exosomes, and their abundance increases under conditions of proteotoxicity. Adding chaperone-containing exosomes to cultured cells expressing aggregation-prone proteins decreases inclusion body formation, demonstrating that exosomes can be an efficient mechanism for the transfer of chaperones between cells (Takeuchi et al. 2015).

In the field of research on neurodegenerative diseases, a series of studies on exosomes have recently been carried out. One of the projects studied neuron-derived exosomes from individuals diagnosed with Alzheimer's disease. The exosomal composition of the patient prior to the onset of symptoms and 10 years after the diagnosis was compared with those from non-affected normal individuals. The results showed that the levels of ubiquitinated and lysosomal proteins were consistently higher in exosomes with Alzheimer's disease, but the abundance of HSC70 was lower (Goetzl et al. 2015). All of these studies have highlighted the potential of exosomal proteins as biomarkers for the diagnosis of neurodegenerative diseases. However, the role of exosomes in aging and age-related diseases, especially in maintaining proteostasis across cells, requires further study (Kaushik and Cuervo 2015).

As previously mentioned, proteotoxic stress in one cell can be transferred to adjacent cells by exosomes. So what does it look like at the tissue or organ level? Recent research shows that, similarly, other tissues would sense local protein damage within one tissue as an integrated organismal response. Van Oosten-Hawle et al. have addressed this question by using myosin temperature-sensitive (ts) mutations expressed only in muscle and observed induction of the myosin chaperone Hsp90 not only in muscle but also in neuronal and intestinal cells. Moreover, cell-nonautonomous expression of Hsp90 in tissues other than muscle suppressed myosin (ts) misfolding at the restrictive temperature. Similarly, instead of expressing Hsp90 in other non-muscle tissue, activating heat shock responses in these tissues also improves folding of myosin mutants in muscle cells. Those observations clearly show that activating the heat shock response in one tissue has a beneficial effect in other tissues. Thus, these results reveal that disruption of proteostasis by expression of metastable muscle proteins generates a muscle-specific stress that is sensed by multiple tissues in the animal and unexpectedly results in a cell-nonautonomous elevated expression of Hsp90. These results reveal a compensatory response to a tissue-specific imbalance in proteostasis that functions in a cell-nonautonomous fashion in the nematode *C. elegans*. Moreover, in the long term, elevated Hsp90 in neurons due to imbalance of proteostasis in muscle tissue inhibits heat shock response in neurons and reduces their anti-stress ability. The consequence of this is that it accelerates the aging of brain tissue and increases the incidence of

neurodegenerative diseases (Van Oosten-Hawle et al. 2013). This means that the aging of other tissues may also accelerate brain aging. Aging of various tissues and organs of the body affects each other in a more intense way than we expected.

6.7 Conclusion

Molecular chaperone plays an important role in maintaining the proteostasis along with UPS and autophagy-lysosome system. During aging, due to oxidative stress and other environmental stress, damaged proteins accumulated inside the neurons formed a sink for chaperones; at the same time, the chaperonin itself is modified at certain residues and results in reduced activity, even causing the chaperone itself to become a toxic protein that damages mitochondrial function. As time goes by, those damaged proteins cause the formation of aggregation. The major player to degrade aggregates in cell is autophagy-lysosome system, which is also compromised in aging neurons, so those insoluble aggregates along with chaperones were expulsed outside the cell through exosomes, cause the spread of toxic protein aggregates to neurons nearby, and further affect other tissues through the pathway observed by Van Oosten-Hawle et al. recently. However, the molecular mechanism of cross talk between different tissues of protein homeostasis regulation is not yet clear, and we still need further research.

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Aging of Human Adult Stem Cells

7

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Abstract

With the continuous development of stem cell research in recent years, it is realized that stem cell aging may be the core issue of organ aging. As an important approach and main content of regenerative medicine, the stem cell research brings great hope to overcome difficult diseases and improve the quality of life for human beings and become the key to solve this issue. Based on this research, the varying characteristics of stem cells in aging could be recognized; the role of stem cells in the organ aging and regeneration will be revealed; the function of stem cells will be controllable and regulatable in tissues and organs; the stem cells from tissues and organs with rapid or slow cell renewal (e.g., liver and neuron) could be continuously observed from the levels of cellular molecules and dynamic complex. With the assistance of systematical research approaches, the function and mechanism studies can be conducted via multi-perspectives and levels during the different stages of organ aging and regeneration. All of the abovementioned requires great efforts to thoroughly understand the basic rule and the way of stem cell regulation in organ aging and regeneration. Final to the end, the dream of antiaging, efficient repair, and organ remodeling could be realized and also can meet the major needs of population health and disease treatment in our country, meaningfully to contribute benefits for the health of human beings.

Keywords

Adult stem cells · Senescence · Organ aging · Regeneration

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7.1 The Stem Cell Aging Might Be the Biological Foundation of the Organ Aging

Cell aging and regeneration are two of the basic biological phenomena during individual development. Cell aging normally occurs in old stage, while regeneration exists all through the life. Under the healthy physiological circumstance, regeneration maintains cellular self-renewal in tissue. Nevertheless, it mainly takes part in the tissues and organs repair under sorts of pathological reasons. In the past decades of years, an increasing knowledge has been obtained on the reorganization of biological characteristics regarding to senescence and regeneration. Especially in recent years, the relationships between stem cells and tissues together with organs in human bodies have been gradually moved to the spotlights and attracted many scientists from different related fields. The clinical and laboratorial studies have demonstrated that stem cell aging might be the biological foundation leading to the tissue and organ aging. It was reported that the aging hematopoietic stem cell in bone marrow is the cellular biological basic of pathophysiological characteristics during the senescence in hematopoietic system, including the decreasing function of immune system and increasing incident of myelogenous diseases (Kollman et al. 2001: Rossi et al. 2008). Besides, other studies suggested the biological characteristics in tissues and organs could be affected by some external factors in stem cell niche. For example, Mayack SR from Harvard University found the serum from young mice can inhibit stem cell senescence in old mice. Meanwhile, they observed insulin-like growth factor-1 (IGF-1) can accelerate cellular senescence (Mayack et al. 2010). Swindell WR group from the University of Michigan Medical School reported a male dwarf mouse, Yoda, which lived over 4 years (roughly the equivalent of a 136-year-old human), still kept remarkable vitality and vigor, and retained a certain fertility. This dwarf mouse was produced by genetic modification that affected his pituitary and thyroid glands, leading his hormone levels significantly low different from the normal ones (Swindell et al. 2009). These studies suggest the following: (1) changing the levels of activated factors or hormones could prevent tissue aging in human organs and even in the whole body; (2) some special factors or components could change the cellular biological characteristic by regulating circulatory systems in the specific tissue stem cells; and (3) the human senescence and regeneration can be intervened or regulated via artificial approaches.

Recently, as the increasing development of studies on neural and liver stem cells, the organ aging and regeneration based on stem cell research have been expending from hematopoietic system to solid organ (e.g., nerve and liver and so on). This forming trend is attributed by the development of two key technologies:

1. The specific tracking methods of genetic markers for stem cells in living tissues. In the past years, it has been observed gradually that some high specific markers existed in tissue stem cells, for example, CD133 and nestin have been applied for the characterization to neural stem cells (Singh et al. 2003), FoxL and Trop2 for liver stem cells (Itoh and Miyajima 2014), $CD45^{-}FLK1^{-}CD105^{+}$ for bone

marrow stem cells (MSCs), and so on (Rubin and Aghamohammadi 2003). Following, those genetic markers could be tracked by Cre-loxP system technically. Therefore, the tissue stem cells and their following regenerations from various developmental stages, physiological and pathological conditions can be separated directly and applied for various studies in the future. Meanwhile, it is possible to observe and analyze cell proliferation, differentiation, migration, and other biological behaviors in the living tissues or organs orthotopically and also to observe the temporality and spatiality of the organized cell renewal or damaged cell repair.

2. The technology of stem cell separation direct from tissues. This technology has been always one of the technical bottlenecks in the research area of stem cell separation. Recently, some special techniques, like microdissection based on high-resolution laser capture, single-cell RNA sequencing, and fluorescence-activated cell sorting, have been successfully applied in tissue stem cell separation (Jaitin et al. 2014).

These developments suggest stem cell separation from various differentiating stages or from their following regenerations in growth could be available to obtain by these mentioned techniques, which also provide more opportunities to study cell aging from different tissues and organs. As the developing technologies applied in cellular biology, molecules and research conditions are available to use on cell and animal work; together with the incorporation of scientific thoughts based on time, space, amount, and integrity, it is believable that the study of organ senescence and regeneration based on stem cells will develop as a new hotspot in the entire bioscience.

7.2 The Present Researches and Advanced Studies

As the stem cell studies go deeper, the permanent loss and self-renewal of stem cells are closely associated with senescence and regeneration. It has been realized that the stem cell aging might be the biological foundation of senescence and regeneration. Therefore, the advanced study on stem cell senescence and regeneration is the basic and bottleneck for antiaging, regulating regeneration and building organs.

In tissue and organ, the senescence and regeneration are two basic biological phenomena during individual development. Senescence occurs in the old stage of the human body. A decreasing response of age-dependent has been linked to the tissues' and organs' atrophy, hypofunction, and decreasing ability to resist disease and repair damage, whereas regeneration appears at every stage of personal development. Under normal biophysical conditions, cells from different tissues and organs are presenting differently in their ability of replication during various biological conditions. The epithelium cells from alimentary canal and respiratory tract display a high regenerative capacity, which is capable to repair damage and also can lead to tumor genesis under pathological conditions. When the human body moves into old stage, the capability of tissue and organ regeneration is gradually

decreasing, accompanied with the rising of age-dependent characteristics. Consequently, the high morbidity increases in some age-associated diseases, like cardiovascular and cerebrovascular diseases, malignant tumor (disorders in regenerative regulation), diabetes, autoimmune disease, repeated infection, impairment of wound healing, and senile dementia. Hence, the issue of senescence and regeneration is not only related with biology but also with medical science of public health.

Senescence and regeneration in tissue and organ became a common concerned study area many years ago. Back to the 1960s of the last century, a plenty of accumulated studies were reported on cell aging (same to cellular senescence), especially for the studies on the biological characteristics and involved mechanism during the process of senescence. In recent years, it has been realized that the stem cell senescence is the biological basis of tissue and organ senescence and regeneration (Ritschka et al. 2017). Correspondingly, the research system has converted from individual observation to an integrated study with multilayer and multi-angle, which conduct scientific research into a new stage of studying mechanism mainly based on a stem cell lineage differentiation in tissues and organs.

Currently, the updated research hotspots are presented as the following aspects.

7.2.1 The Correlation Between Stem Cell, Tissue, Organ, and Body Senescence or Regeneration

It has been widely accepted that almost every tissue in the body contains various types of stem cells, which exist in a dynamic balance of stem cell pools that maintain a dynamic balance in tissue or organ structures and functions. Additionally, the senescence in tissues and organs normally happened with a decrease in the turnover of cellular components. The most common view at present is that the key factor leading the decrease of cellular turnover is related with the dysfunction of stem cells. Some studies have identified the trend of age-dependent decrease in biological function in tissue stem cells. For example, Kollman C analyzed 6978 patients who had undergone bone marrow transplantation and observed that the patients receiving bone marrow from older donors had more complications which resulted in the lower success rate (Kollman et al. 2001). This study demonstrates that the decrease ability of human bone marrow proliferation and differentiation is age-dependent that suggests this decreasing ability might be intrinsically regulated by the stem cell itself. The similar phenomenon was also confirmed in mouse, where a research model was set up for studying the relationship between the senescence of hematopoietic stem cells and related diseases (Rossi et al. 2008). In the liver, it was detected the chromosome ploidy of hepatocyte in hepatic lobule exhibits an increasing trend from the portal area to central veins. The hepatocytes accumulated near the portal area are generally diploid cells, and the ones near the central veins present tetraploid or even octaploid cells. This phenomenon strongly suggests the senescence occurred in hepatocytes of hepatic lobule shows a sort of hierarchy and directionality from spatial structure. Therefore, this recognition that hepatocytes derived from portal

area support those stem cells that form structural basis for cell turnover in tissue and organs.

In the central nervous system, the ability of neural generation is going down as age is increasing. It was observed that the cancer suppressor gene $p16^{ink4a}$ could be activated during the senescence of nervous tissues in mice (Skowronskakrawczyk et al. 2015). Thereafter, $p16^{ink4a}$ might be used as one of the biological markers in aging nervous system. The upregulation of $p16^{ink4a}$ was observed in aging nervous tissue and proves to reduce the self-renewal of neural stem cells, which suggests neuron stem cell aging might be one of the biological basics in nervous tissue senescence (Molofsky et al. 2006). Besides, studies on mitochondrial metabolism, telomere function, and the characteristics of proliferation and differentiation also demonstrated that tissue stem cells and somatic cells both exhibit senescence phenomenon.

7.2.2 The Stability of Genetic Structure in Tissue Stem Cells

From the consideration of cellular endogenous factors, once the body with gradually sliding into a senescent state, the accumulation of DNA damage that happened inside tissue stem cells becomes obvious to detect and results in DNA damage response (DDR). It was reported that DDR is an unavoidable happening during cellular senescence and might be a "mutual pivot" of various endogenous factors which lead to senescence in stem cells (Niedernhofer et al. 2006; di Fagagna 2008; Murga et al. 2009). As known, many exogenous physicochemical factors like ionizing radiation and endogenous factors like reactive oxygen can cause different kinds of DNA damages, including 8-oxo-G and DNA double-strand breaks (DSBs). It has been accepted that the accumulation of DNA damages can result in stem cell exhaustion, which continuously causes tissue, organ, and final body senescence. Additionally, animal studies have revealed that caloric restriction (CR) can lower the level of reactive oxygen species, extend lifespan, induce telomere loss during cellular senescence, and so on. All these functions are effectively related with DDR mechanism. Furthermore, the mouse model of progeria also identified that the DDR mechanism is the key factor leading cells into senescence, rather than the result of this phenomenon. For example, the deficient mouse with the deletion of DNA damage repair pathways like Ku80 and Lig4 presents serious progeria symptoms. The Ku80 works as a gatekeeper of nonhomologous end joining protein in vivo. The deficiency of Ku80 exhibits hepatocyte degeneration with the detection of inclusion body and heterochromatin; the Lig4 deficiency can accelerate the exhaustion of hematopoietic stem cells (Fig. 7.1). These above evidences suggest that the DNA damage accumulation observed in stem cells from tissues and organs could cause stem cell senescence and further lead to abnormal characteristics during stem cell renewal under various biophysical states. It has been generally regarded that the endogenous event like DNA damage and cellular response is one of the significant research points for studying the related mechanism in tissue and organ senescence and regeneration (Lebel et al. 2011).

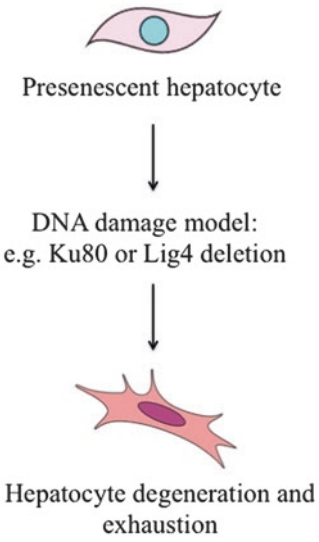

Fig. 7.1 The deletion of DNA damage repair pathways like Ku80 and Lig4 can lead to hepatocyte degeneration and exhaustion

7.2.3 The Regulation of Epigenetics in Tissue Stem Cells

The epigenetic modification in various systems (including neural system, hematopoietic system, liver system, etc.) plays an important role in senescence, cytopathy, tissue repair, and generation. A remarkable study from Jeanisch's group showed that DNA methylation exhibits a crucial effect in regulating neurons and lifespans. Although the knockout mice with a deletion of DNA transmethylase Dnmt3a grew healthily after being born, the neural system was gradually degenerated since adult stage and finally suffered from premature death (Nguyen et al. 2007). However, it has not been clear whether neural stem cells can be effected or not in that above senescence study model. During the senescence observed in old mice, miRNA let-7b can reduce the expression of high mobility group AT-hook 2 (HMGA2), which subsequently reduces the ability of self-renewal in neural stem cells (Nishino et al. 2008). This indicates miRNA is one of the regulators in tissue stem cell behaviors. It has been known that miRNAs can mediate the stem cell cycle and the secretion of cellular matrix or growth factors that further affect individual body senescence and lifespan. Our study found the selective knockout of Dicer (an enzyme to produce mature miRNA) in astrocytes resulted in mice death after being born in 2 months. Meanwhile, these mice presented cerebellar atrophy and degeneration of neuron in other encephalic regions. This phenomenon suggests miRNAs take part in the regulation of neural senescence and generation, which provides us a hint, namely, the observation and analysis for the changes of neural stem cells might further reveal the various regulating approaches from microenvironment working on stem cells in epigenetics. Among the patients with Alzheimer's disease (AD), the methylation level of CpGs from some genes shows significantly increased expression compared with healthy ones (De Jager et al. 2014). Some of these genes are related with neural

development and central nervous system (CNS) tumorigenesis. Additionally, under natural state, the senescence of hematopoietic stem cells is most likely regulated by epigenetics, which leads the increasing potential of myeloid cell differentiation, the upregulating expression of myeloid-specific genes and leukemia genes, and finally the increasing incidence rate of osteal diseases (Kim et al. 2008). Besides, the differentiation potential of lymphocytes and expression level of lymphocyte-specific genes might be downregulated that consequently result in the reduced ability of the immune system. In addition, it was also observed that the appropriate expression of telomere is one of the necessary conditions to maintain the ability of mesenchymal stem cell (MSC) proliferation. This process might be regulated by epigenetics as well. These studies indicate epigenetics acts a crucial function in regulating the normal and abnormal differentiation caused by senescence in tissue stem cells.

7.2.4 The Interactions of Microenvironment and Tissue Stem Cells

The structure of microenvironment can maintain the capability of stem cell self-renewal and has been found in various tissues including the epidermis, alimentary canal, neural system, liver, gonad, and others. This structure consists of niche cells, cellular matrix, and secreted soluble factors. The microenvironment can generate multiple biological functions via direct and indirect interactions with stem cells. Some changes from microenvironment can reduce the nutrition supplement of the local stem cells and also can inhibit the interactions to the external or become low sensitive in the same reaction. For example, some factors like bioactive molecules and extracellular matrix around neural stem cells can support their pluripotent characteristics and also can regulate stem cell differentiation (Gattazzo et al. 2014). For the liver stem cells, the changes that happened in microenvironment factors like cell growth factors, extracellular matrix, epidermal cells, and parenchymal cells can regulate liver stem cell signaling pathways, such as MAPK, PI3K, Wnt, integrin, and so on (Suzuki et al. 2003; Mavila et al. 2012), to further affect the stem cell activation and differentiation. Besides, it has been revealed that the microenvironment can act in the spatial migration of stem cells and local wound repair. Previously, the study demonstrated that the stem cell migration and residence in the target organs are the important prerequisites for their essential function in vivo. In graft-versus-host disease (GVHD) study, we also observed the correlation between the changes of expression level for cellular surface chemokine receptor and disease occurrence (He et al. 2008).

As for the functionality of a microenvironment in tissue repair, the differentiation-inducing growth factors can trigger some specific differentiation procedures in tissue stem cells, followed by the differentiation to the end stage. Therefore, they participate in the organizational remolding and functional reconstruction of tissues. Similarly in our study, we observed that with the treatment of sphingosine 1-phosphate (S1P), bone marrow mesenchymal stem cells showed dramatic migration towards the damaged liver tissues, and differentiated into fibroblasts, thus

accelerating liver fibrosis (Li et al. 2009). Nevertheless, the exact mechanisms regulating stem cells differentiation into fibroblasts in tissue microenvironment remain unknown.

The micronutrient in microenvironment has been reported to regulate the functional activities of tissue stem cells. Our previous study found the lack of zinc showed significant inhibition in the function of neural stem cells (Di Wang et al. 2001). The deletion of Slc39a11, Slc39a12, and/or iron metabolism-related TGF-β lacking phenotype in knockout animal models, will benefit the further study to analyse the mechanism of how zinc ion, iron ion, and TGF-β regulate stem cell activation, generation, and differentiation destiny in aging organs (Massagué and Xi 2012; Lin et al. 2013).

7.2.5 The Regulation of Exogenous Stem Cells in Senescence and Generation

The present study proposes that the majority of tissue stem cells are low-immunogenic cells with no or low expression of major histocompatibility complex (MHC) and co-stimulating molecules. With the cellular differentiation occurring in vivo, those expressions of immune-related molecules are increased, followed by the enhancement of immunogenicity and activation of immunoreaction. Plenty of cytokines in recipient's microenvironment can change the immunological characteristics of exogenous stem cells, and the implanted stem cells also can conversely change the recipient's immunological function (Jin et al. 2008). Therefore, the study for the expression profiles from different stages of tissue stem cells and the setup for the identification and specific markers of immune functions are of great significance to regulate stem cell performance. Since 2004, MSC was found as a sort of immunosuppressive cells that have been used for GVHD treatment. These stem cells with low toxic and side effect exhibit a promising perspective. It is grateful to notice that this area of research in our country is running at the top of the world. We firstly observe that MSCs transplantation can inhibit GVHD occurrence, because MSC could be induced secretly to many kinds of chemotactic factors under the stimulation of specific inflammatory factors in vivo, together with the generation of other suppressive factors including nitric oxide (NO), indoleamine 2,3-dioxygenase (IDO), and others (Zhu et al. 2006). Recently, another study reported the inflammatory factors released from injured tissues could stimulate MSCs to generate cell growth factor, which contributes to the tissue repair (Xu et al. 2009). This study further indicates that the mechanism of MSC-mediated regulation and differentiation could be benefical for setting up an intervention system based on using MSCs for studying senescence and generation. Thereafter, this system will be helpful to discover new targets in intervention and regulation, meanwhile to provide novel ideas for immunoregulation.

Although some knowledge has been accumulated to recognize the correlation between tissue stem cells and organ senescence or generation, the recognition is still basically collected from single case or event. According to the practice, we tend to the view that the advanced studies in tissue and organ senescence will definitely

apply the concept of "space-time," the mainline of stem cell differentiation profile, and the tri-directions of study subjects (tissues or organs, cells, and molecules), to reveal the biological mechanism that exists in stem cell aging, aiming to find out more key molecules that regulate stem cell activation, targeting, and differentiation. Moreover, the studying points and key issues to be solved might focus on the following three aspects: (1) the changing characteristics in stem cell aging, (2) the development regularity of stem cells during organ senescence and generation, and (3) the regularability of stem cells in organ senescence and generation. These issues are the keys to better understand the occurrence mechanisms of spatiotemporal behaviors during stem cell differentiation profile. Based on these strategies, the final aims will be achieved in the recognition of the internal regularity of stem cell-regulated senescence and generation, meanwhile to provide a foundation for the future artificial controls or intervention, even for the further interpretation in every senescence and generation.

We believe that the development of advanced studies in stem cell senescence and regeneration will absolutely guide and promote other related medicine fields including stem cell-based regenerative medicine to explore more possibilities.

With the consideration of the present conditions and advantages, we propose that the priority strategy in performing stem cell studies in regard to senescence and generation should involve the following aspects: using animal model, targeting specific genetic markers in vivo, comprehensively analyzing spatiotemporal behaviors from targeted tissue stem cells during their senescence and regeneration in different stages and perspectives, and discussing the regulatory mechanism and internal rules rising in the whole processing stages. This way of study is aiming to solve scientific issues from the following three aspects:

1. What will happen in the changes from "quality" and "quantity" of stem cells during individual senescence, moreover whether the changes could present some certain of specific markers or morphological characteristics?
2. What is the internal correlation between tissue stem cells and organ senescence/generation with spatial and temporal changes? And what is the molecular basis and involved key regulators during the mediating process?
3. What kind of external factors could be accepted for organs and tissue stem cells? Furthermore, we hope that deeper understanding in answering these questions can provide a new theoretical basis for the research of stem cell-related biomedical problems such as the prevention and treatment of various types of cancer and senile diseases.

From what has been discussed above, we believe that the rise and development of tissue stem cells in the field of senescence and regeneration research will affect the whole trend of biomedical studies. Since we are now in this new frontier of this field, we should seize this rare opportunity, to develop this area as soon as possible, to produce a batch of the landmark result of the original, to inject new power for the high level of related biological medicine, and also to prepare benefit conditions for conquering the advantage subject status of this field in a new round of competition.

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8 Mechanisms of Hematopoietic Stem Cell Ageing and Targets for Hematopoietic Tumour Prevention

Min Liao and Jianwei Wang

Abstract

Hematopoietic stem cells represent a rare population in the bone marrow, with the capacity of generating all blood lineage and themselves at the same time. With aging, the reconstitution capacity of hematopoietic stem cells decreases accompanying with differentiation skewing wherein the myeloid branch dominates in both mouse and human. In recent years, various molecular mechanisms that induce functional decline of HSC during aging were disclosed including DNA damage accumulation, metabolic alteration, defects in protein homeostasis, and aging-induced changes in the blood circulatory environment. Deciphering the nature of HSC aging could improve our knowledge of HSC aging-related diseases and furthermore promote the developing of therapeutic interventions for human HSC aging and diseases.

Keywords

Hematopoietic stem cell · Aging · Self-renewal · Differentiation · Leukemia

8.1 Introduction

As a part of life, aging results in the deterioration of tissue regeneration and homeostasis. The aging-associated decline in the functionality of tissue-specific stem cells contributes to this phenomenon. Stem cells are present in almost all somatic tissues of adult humans, and although they are rare in number, this rare population of undifferentiated cells is thought to be required for lifelong maintenance of organ integrity (Goodell et al. 2015; Adams et al. 2015; Sousa-Victor et al. 2015; Ortells and Keyes

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2014; Biteau 2015). Aging-associated defects in stem cell function appear in a tissue-specific manner. For example, muscle aging is characterized by the loss of muscle mass and strength reflected by a decrease in the number and function skeletal muscle stem cells (also known as satellite cells) with aging (Sousa-Victor et al. 2015). Aged muscle stem cells exhibit defect in differentiation characterized by an increase potential to differentiate into fibrogenic lineages, resulting in tissue fibrosis (Brack et al. 2007). In skin, aging-associated phenotypes include the loss of hair, dermal thinning, and constricted wound healing. Although changes in the frequency of skin stem cells with aging remain under debate, the function of skin stem cells clearly declines upon aging (Ortells and Keyes 2014). Even though the changes in stem cell number with aging are tissue-specific, functional decline during aging is a common feature between skeletal muscle and skin stem cells. Hematopoietic stem cells (HSCs) reside in quiescence only getting activated every 3–5 months (Wilson et al. 2008), and an in vivo labeling assay shows that the net proliferation of HSCs is ~1% per day (Busch et al. 2015), indicating that the balance between differentiation and self-renewal of HSCs is fine-tuned at young age. With aging, the balance appears to be disturbed as the number of phenotypically defined HSCs (based on surface markers) increases, but the repopulation capacity of HSCs declines, and the hematopoietic output is skewed toward myeloid lineage in aging mouse and human (Beerman et al. 2010; Pang et al. 2011). These defects result in impairments in lymphopoiesis likely contributing to the emergence of defects in adaptive immunity during aging (Wang et al. 2011). There is experimental evidence that this aging-associated skewing in hematopoiesis is to some extent due to a drift in the pool of HSCs itself (Busch et al. 2015; Muller-Sieburg et al. 2004). Apparently the pool of HSCs consists of different subpopulation of HSCs including lymphoid-biased HSCs and myeloid-biased HSCs among other types (Busch et al. 2015; Morita et al. 2010). Aging associates with increases in the number of myeloid-biased HSCs, whereas the number of lymphoid-biased HSCs remains largely unchanged, which results in a relative increase in the percentage of myeloid over lymphoid-biased HSCs. Additional factors may influence myeloid-lymphoid skewing in aging, such as cell intrinsic changes in differentiation capacity (Rossi et al. 2005) as well as aging-induced alteration in the stem cell niche or the blood circulators environment observed in response to telomere shortening (Song et al. 2010; Ju et al. 2007). Together these defects of aging HSCs likely contribute to the evolution of aging-associated hematopoietic diseases and malignancies such as anemia (Guralnik et al. 2004) and myeloproliferative diseases (Lichtman and Rowe 2004). The uncovering of molecular mechanism of HSC aging will likely help the future development of new therapies aiming to prevent functional defects of aging HSC and thus the development of hematopoietic diseases. In this review, we will summarize recent progress in HSC aging and highlight the effect of HSC aging on hematopoietic disorders.

8.2 Microenvironment, Systematic Factors, and HSC Aging

The bone marrow is a natural shelter to foster hematopoietic stem and progenitor cells, which is called microenvironment or niche. Transplantation experiments indicate that the aged microenvironment deteriorates HSC function, which contributes to aging-associated myeloproliferative disease and influences clonality of hematopoiesis (Vas et al. 2012a, b). Young HSCs engrafted in aged mice show a compromised proliferation potential and a declined differentiation toward lymphocyte compared to that growing in young recipients. Vice versa, old HSCs engrafted in young microenvironment show ameliorated reconstitution capacity and balanced differentiation potential (Ergen et al. 2012). Various cell types including endothelial cell, perivascular cell, osteoblast cell, mesenchymal stem cells (MSCs), sympathetic nerve cells, adipocytes, macrophages, and nonmyelinating Schwann may contribute to maintain HSC self-renewal, differentiation, and proliferation (Boulais and Frenette 2015; Morrison and Scadden 2014; McCabe et al. 2015) (Fig. 8.1). Although great progress has been made in understanding the function of the HSC niches, the influence of niches on HSC aging remains largely unknown. The mobilization efficiency of aged mice is five times higher than that of young mice, and decrease in the adhesion of HSCs to stromal cells and elevations in the levels of GTP-bound Cdc42 have been implied to contribute to this phenotype (Xing et al. 2006). Several experiments indicated that alterations in genes that regulate cell adhesion influence HSC function. For example, Cdc42, a small GTPase of Rho subfamily, is a critical coordinator regulating HSC interaction with the bone marrow (BM) niche. Several key adhesion regulators are disturbed in Cdc42-deficient HSCs (Yang et al. 2007). Constitutively elevated Cdc42 promoted a premature aging phenotype of HSCs characterized by loss of repopulation capacity, differentiation skewing, and decreased cell polarity (Florian et al. 2012). Osteopontin (OPN) in the murine bone marrow stroma declines with age and results in the decrease of reconstitution capacity of HSC accompanying with enhanced myelopoiesis and loss of HSC polarity (Guidi et al. 2017). Moreover, Wnt5a-haploinsufficient niche regenerates dysfunctional HSCs mediated by Cdc42-related apolar F-actin localization (Schreck et al. 2017). Conflicting study shows that Wnt5a maintains HSC integrity by modulating canonical Wnt signaling (Nemeth et al. 2007). However, intrinsic haploinsufficient Wnt5a attenuated aged HSCs (Schreck et al. 2017). A recent study implicates that inhibition of Wnt signaling rescues the function of Sirt6-deficient HSCs, wherein a series of Wnt-transcribed genes were found upregulated (Wang et al. 2016). These findings suggest a comprehensive role of extrinsic or intrinsic Wnt5a in regulating HSC function. EphB4, a tyrosine kinase receptor, mediates the interaction between niche and HSCs, and overexpression of EphB4 was shown to promote HSCs (Nguyen et al. 2015). Interestingly, transplanted HSCs showed enhanced engraftment potential when transplanted into EphB4 transgenic mice compared to wild-type mice indicating that EphB4 enhances HSC niche functions to promote HSC engraftment (Nguyen et al. 2015). Whether EphB4 expression changes in aging or whether overexpression of EphB4 would improve the functionality of HSCs niches in aging bone marrow remains to be investigated. The

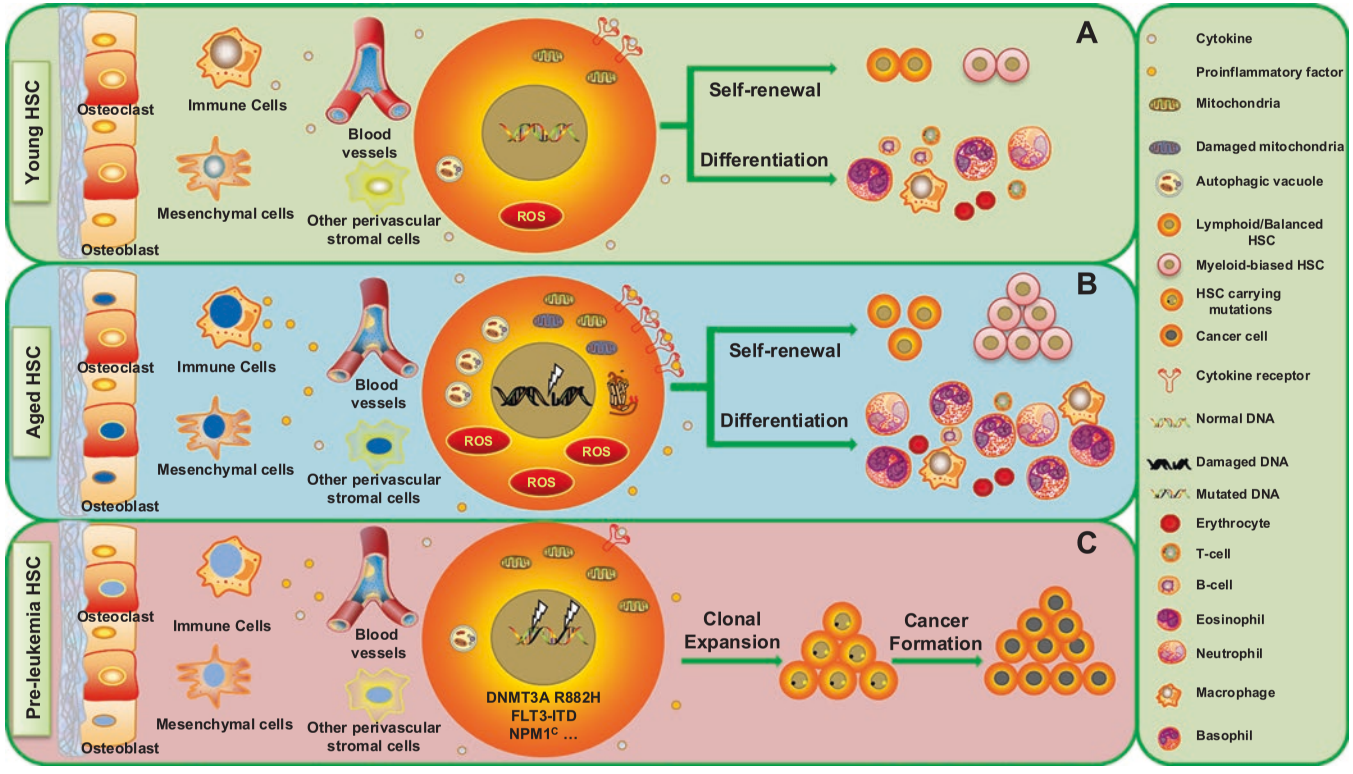

Fig. 8.1 Representative schema illustrates the intrinsic and extrinsic alteration of HSC in different stages: young, old, and preleukemic. HSCs locate in a particular niche surrounding with supporting cells and generate all of the blood cells and itself, which is called self-renew (**a**). The quality and quantity of supporting cells and HSC itself change with aging (**b**). These changes may predispose HSC to a preleukemic stage (**c**)

interaction between stem cell niches and HSCs could represent a promising therapeutic target to improve the function of aged HSCs.

Not only local environment but also systematic alteration influences HSC aging. Wild-type (WT) HSCs transplanted into telomerase-deficient mice with short telomeres show impaired maintenance of HSC quiescence and a decrease potential to differentiate into lymphoid lineages (Song et al. 2010, 2012; Ju et al. 2007). Transplantation of bone marrow and thymi revealed that telomere dysfunction-induced impairments in lymphopoiesis were mainly due to alterations in the systemic blood circulatory environment rather than alterations of the niche. These data indicate that DNA damage induced by telomere dysfunction deteriorates the environmental function. One of the factors that appeared to contribute to this process is granulocyte-colony stimulating factor (Gcsf), which increases in serum of telomere dysfunctional mice (Ju et al. 2007). Indeed, Gcsf treatment was shown to impair HSCs differentiation into lymphocyte lineage in WT mice (Ju et al. 2007), which may involve the known inhibitory effects of Gcsf on B lymphopoiesis by suppression of B cell trophic factors through reprogramming of stromal cells (Day et al. 2015).

Several lines of evidence indicate that the increase in the production of pro-inflammatory cytokines and growth factors could be a general mechanism contributing to aging-induced defects in HSC function. Deletion of retinoic acid receptor gamma (RARγ) results in impaired microenvironment, inducing hematopoietic disorders mediated at least in part by elevated TNFα expression (Walkley et al. 2007). TNFα compromises the function of HSCs by enhancing commitment to the myeloid lineage, a phenotype of HSC aging (Dybedal et al. 2001). Whether these circuits contribute to natural aging remains to be delineated. Interestingly, TNFα is a component of the senescence-associated secretory phenotype leading to an increased expression of several pro-inflammatory cytokines (Franceschi and Campisi 2014). Several of these factors increase in response to telomere dysfunction-induced aging as well as during human aging (Ju et al. 2007; de Gonzalo-Calvo et al. 2010; Daynes et al. 1993). It is conceivable that activation of TNFα and other pro-inflammatory cytokines contributes to the declines in HSC function during aging. Consistent with this, application of IL1β predisposes HSC to aging-like phenotype (Pietras et al. 2016). The inflammatory cytokine, chemokine ligand 5 (Ccl5, also called Rantes), increases in aged stem cell milieu, which results in HSC skewing to myeloid-biased differentiation. Interestingly, Rantes-deficient mice showed a reversal of HSC differentiation defects during aging. However, the Rantes genotype did not influence HSC function in transplantation experiments suggesting that Rantes affects HSC aging through stem cell extrinsic, niche/environment-dependent effect (Ergen et al. 2012). Together, the discussed studies suggest that a variety of pro-inflammatory factors and growth factors contribute to defects in HSC function and differentiation during aging. It would be of interest to investigate the effect of inhibitory compounds that target these systemic acting factors to reverse defects of aging HSCs.

In addition to its effects on HSC differentiation, inflammatory factor may influence HSC self-renewal. Nestin^{+} mesenchymal stem cells (MSCs) were reported to be spatially located with HSC and to assist the maintenance of HSCs by expressing

HSCs' maintenance-related genes. Of note, Gcsf stimulation induces a downregulation of HSCs' maintenance-related genes in MSCs resulting in the loss of HSCs (Mendez-Ferrer et al. 2010). The function of MSCs declines with aging in mice and humans (Bergman et al. 1996; Stenderup et al. 2003). It is tempting to speculate that alterations in MSC function may impact on HSC functionality during aging. Along this line, a recent study showed that SHIP1-deficient MSCs promote HSC differentiation toward myeloid branch and SHIP1 prevents Gcsf production by the aging MSC compartment (Iyer et al. 2015). Taken together, aging-induced dysfunction of MSCs could contribute to the evolution of aging-induced defects in HSC function and the development of hematopoietic diseases. Interestingly, myelodysplastic cells of myelodysplastic syndrome (MDS) patient were recently shown to reprogram MSC to a niche cell propagating myelodysplastic cells (Medyouf et al. 2014). These findings indicate that the interaction between MSCs and disease initiating cells of the hematopoietic system could be bi-directional.

All the above evidence indicates that impairments in the BM niche and altered systematic signals influence HSC proliferation, self-renewal, and differentiation potential. However, how this interaction between niche, systemic factors, and HSC influences HSC function during aging requires further investigation, and such knowledge would hold the promise to develop therapies aiming to improve HSC function and hematopoietic disease prevention in the elderly.

8.3 DNA Damage Accumulation and HSC Aging

HSCs isolated from aged wild-type or telomerase-deficient mouse appear to accumulate significantly elevated amounts of DNA damage (Rossi et al. 2007; Mohrin et al. 2010; Wang et al. 2014a, b; Beerman et al. 2014) as revealed by γH2AX foci, a marker of early responses at DNA double-strand breaks, or by the comet assay – a method to directly detect DNA breaks in HSC. Some of the DNA damage markers are still under debate, and the development of new assay would clearly be beneficial. However, next-generation sequencing analysis revealed clear evidence that clonal hematopoiesis originating from mutant stem or progenitor cell increases during aging (Xie et al. 2014) and the emergence of clonal hematopoiesis predisposes aging individually to the development of hematologic disease, cancer, and death (Jaiswal et al. 2014; Genovese et al. 2014). Together, these data indicate that DNA damage and mutations accumulate in HSCs and this leads to HSC dysfunction and disease development.

Several factors have been identified that can contribute to the accumulation of DNA damage and mutations during aging including telomere shortening (Rossi et al. 2007; Choudhury et al. 2007), replication stress (Flach et al. 2014; Alvarez et al. 2015), proliferation-induced oxidative stress (Walter et al. 2015), and endogenous mutagens such as aldehydes (Langevin et al. 2011). There is increasing knowledge on mechanisms that protect HSCs from accumulation of DNA damage.

8.3.1 Quiescence Protects from Damage Accumulation

HSCs have the capability to maintain cell cycle quiescence, which is interrupted by rare rounds of cell divisions (Wilson et al. 2008). HSC quiescence is also associated with low rates of metabolism (Simsek et al. 2010). Both the reduction in cell division rates by itself and the lowering of metabolic activity may contribute to the protection of quiescent HSCs to accumulate DNA damage resulting in functional decline of HSCs during aging (Rossi et al. 2007; Choudhury et al. 2007). Indeed, defects in the maintenance of HSC quiescence in telomere dysfunctional mice were associated with impairments in maintenance of HSC function (Song et al. 2012). In addition, it was shown that reactive oxygen species (ROS) sharply increase when HSCs are called into cell cycle and this ROS burst leads to DNA damage, which aggravates bone marrow failure in DNA repair-deficient mice (Flach et al. 2014). While maintenance of quiescence has protective effects, it may also have some adverse effects to be considered. Along these lines, it was shown that quiescence renders HSCs susceptible to accumulate DNA damage by employing error-prone nonhomologous end joining (NHEJ) pathways for DNA repair (Mohrin et al. 2010).

8.3.2 DNA Damage Checkpoints and Repair Prevent the Transfer of DNA Damage from Stem to Progenitor Cells

When called into cell cycle, HSCs that harbor DNA damage produce progenitor cells that carry considerably lower amounts of DNA damage (Wang et al. 2014b). Mechanisms that prevent the transition of damage from stem cells to progenitor cells include the induction of senescence or apoptosis (Wang et al. 2014b; Flach et al. 2014) as well as the repair of damaged DNA in response to cell cycle entry (Beerman et al. 2014). In addition, a new type of checkpoint was identified, which limits the self-renewal of HSCs in response to DNA damage by inducing differentiation (Wang et al. 2014a). This checkpoint was dependent on the transcription factor Batf, which modulates Ap1-dependent transcription. Whether the above-described checkpoint responses prevent the clonal expansion of mutant HSCs or the induction of leukemia remains to be delineated. An ATM-dependent differentiation-inducing checkpoint was shown to push melanocytic stem cells into terminal differentiation indicating that this type of checkpoint may be a general mechanism protecting different types of stem cells from DNA damage accumulation (Inomata et al. 2009). Interestingly, DNA damage accumulation mediated by Mll4 deletion constrains leukemogenesis initiated by MLL-AF9 oncogene by inducing ROS-dependent differentiation, which was ameliorated by ATM. These data indicate that DNA damage-induced differentiation can also protect the transformation of HSCs into leukemic cells and the role of specific genes in differentiation induction or prevention can be context dependent (Santos et al. 2014).

The role of Batf-dependent induction of HSC differentiation in tumor suppression is currently unknown. In addition to its potential positive effects on the suppression of DNA damage and tumor formation, Batf-dependent checkpoint function

may also contribute to the loss of lymphoid-biased HSCs (Ly-HSCs) and hematopoietic skewing during aging. Interestingly, Batf-dependent induction of HSC differentiation in response to DNA damage was most active in Ly-HSCs leading to preferential depletion of Ly-HSCs in the context of DNA damage. As discussed above, aging of the hematopoietic system is characterized by an accumulation of DNA damage and a skewed differentiation resulting in a reduced differentiation potential to lymphoid lineage, which likely contributes to the compromise in immune functions with aging (Wang et al. 2011). Several studies showed that the SLAM marker CD150 (Kiel et al. 2005) sub-fractionates HSCs into My-HSCs and lymphoid-biased HSCs (Ly-HSCs) (Beerman et al. 2010; Morita et al. 2010). With aging, My-HSCs dominate the stem cell pool (see above and 9). It is possible that the activation of the Batf-dependent, differentiation-inducing checkpoint contributes to the loss of Ly-HSCs and hematopoietic skewing during aging.

Deletion of p53 impairs the removal of genomic instable intestinal stem cells in aging telomere dysfunctional mice and results in shorter life span compared to telomerase-deficient mice (Begus-Nahrmann et al. 2009). Along with this, p53 coordinates with Aspp1 to protect HSC integrity by limiting DNA damage accumulation, and p53 is required for differentiation induction of HSC in response to Batf activation (Santos et al. 2014; Yamashita et al. 2015). These studies indicate that activation of p53 is indispensable to keep genome stability by removing genetically instable stem cells. Telomerase-deficient HSCs exhibit compromised function in self-renew and rebuilding blood system by activating cyclin-dependent kinase inhibitor 1A (p21) and BCL2-binding component 3 (Puma), which are two important downstream targets of p53. Targeted deletion p21 or Puma rescued intestine stem cell and HSC function (Choudhury et al. 2007; Sperka et al. 2011) and extended life span of telomere dysfunctional mice, indicating that telomere dysfunction deteriorates stem cell function by activating p53-dependent checkpoints. Moreover, application of ABT263, a specific inhibitor of BCL2 and BCL-XL, an anti-apoptotic proteins involved in p53 signaling, selectively kills senescent cells and rejuvenated aged HSCs in normally aged mice (Chang et al. 2016). These studies suggest a dual role of p53 in regulating stem cell function during aging, on the one hand protecting from the accumulation of damaged stem cells and on the other hand contributing to stem cell exhaustion and tissue dysfunction. The effects of p53 appear to be gene dose dependent. HSCs isolated from $p53^{+/-}$ mice proliferate faster than that of age-matched wild-type mice but depict a premature acquisition of aging-associated gene expression changes. In contrast, p53 hypermorphic ($p53^{+/m}$) HSCs display a longer maintenance of a youthful gene expression signature but prematurely exhaust in terms of HSC maintenance and function (Dumble et al. 2007). The effects of p53 gene dosage on HSC aging appear to be of physiological relevance as there is experimental evidence for aging-induced loss of p53 induction in cells of the hematopoietic system in response to DNA damage (Feng et al. 2007).

8.4 Metabolites in Aged Hematopoietic Stem Cells

A large fraction of energy production in eukaryotic cells depends on aerobic metabolism in mitochondria providing most of the ATP for cellular reactions. Mitochondria metabolism influences the function of HSCs, and there is emerging evidence that it contributes to HSC aging.

Perturbation in mitochondria impairs HSC function (Norddahl et al. 2011). It was shown that SIRT3 supports the metabolic homeostasis of mitochondria in HSCs but SIRT3 levels decline during aging (Brown et al. 2013). Interestingly, SIRT3 overexpression could ameliorate aging-induced defects in HSC function indicating that mitochondria dysfunction contributes to the functional decline of HSC during aging. Telomere shortening represents another mechanism that could contribute to aging-induced decline in mitochondria function. Studies on telomerase-deficient mice revealed experimental evidence that telomere dysfunction suppresses the expression of peroxisome proliferator-activated receptor gamma coactivator 1 alpha and beta (PGC-1α and PGC-1β) and both factors are needed to maintain mitochondrial biogenesis and function (Sahin et al. 2011). Of note, overexpression of the PGC1 homolog (dPGC1/spargel) in *Drosophila* was sufficient to prolong mitochondrial activity and the overall life span of the *Drosophila* suggesting that decreases in mitochondrial contribute to aging and longevity (Rera et al. 2011). Of note, glucose supplementation was sufficient to prolong organ maintenance and life span of telomere dysfunctional mice indicating that the maintenance of energy homeostasis and organ functions in aging may require an adaptation of dietary regiments including a higher content of glucose in the context of telomere dysfunction-induced aging (Missios et al. 2014).

Mitochondrial activity in HSCs is relatively low (Ito et al. 2016), which is reflected by low reactive oxygen species (ROS) activity in HSCs while higher in committed lineages (Simsek et al. 2010; Inoue et al. 2010). The low rate of mitochondrial and overall metabolic activity in HSCs is thought to contribute to the protection of HSCs from damage accumulation. By-products of mitochondrial metabolism include ROS and reactive nitrogen species (RNS). It was shown that RNS promotes HSC proliferation and differentiation into the myeloid branch (Nogueira-Pedro et al. 2014). Compared to RNS, ROS is the main by-product of aerobic metabolism through oxidative phosphorylation in mitochondrial electron transport chain (Wallace 2005). Moderate and transient increases in ROS levels exert beneficial effects on cell or organismal aging – in agreement with the concept of hormesis (Macip et al. 2003; Yee et al. 2014; Lee et al. 2010; Schulz et al. 2007). In contrast, constant or high levels of ROS elevation appear to deteriorate cellular and organismal functions (Willems et al. 2015).

It has been postulated that HSCs reside in a hypoxic status with low metabolic activity, which protects HSCs from ROS-induced damages but may also confer HSCs sensitive to ROS elevation (Fig. 8.1). However, it is still under debate whether the HSC niche as such is providing a hypoxic environment or the entire bone marrow is hypoxic due to the high cell cycle activity of hematopoietic cells (for review see 23). In line with the hypothesis that HSCs reside in a hypoxic environment, it

has been shown that HSCs are sensitive to hypoxia-dependent cytotoxic drugs (Parmar et al. 2007) and elevation of ROS in both humans (Yahata et al. 2011) and mice (Ito et al. 2006). Brief exposure of HSCs to ambient air (containing 21% O2) deteriorates long-term reconstitution capacity by increasing ROS activity mediated by the CypD-p53-MPTP signaling axis (Mantel et al. 2015). HSCs with low ROS exhibit higher reconstitution potential than HSCs with high-level ROS. Moreover, HSCs with high levels of ROS show myeloid skewing, thus mimicking the differentiation skewing observed in aged mice (Jang and Sharkis 2007). Overproduction of ROS limits HSC function, but this effect was rescued by NAC (N-acetyl-L-cysteine) administration (Ito et al. 2006). Pharmacologic inhibition of ROS by NAC or rapamycin leads to simultaneous inhibition of other aging-related pathways, such as mTOR or p38 which is recently identified to activate purine metabolism to call HSPC into cell cycle upon stress (Karigane et al. 2016), thereby restoring the reconstitution capacity of HSCs with high ROS (Ito et al. 2006). In addition, deletion of Tsc1, a negative regulator of mTOR activity, induces ROS and mitochondria dysfunction, furthermore compromising HSC function and premature aging of HSCs (Chen et al. 2008). Treatment of Tsc1-deficient HSCs with rapamycin or NAC rescues the decline of HSCs, indicating that Tsc1-mTOR is a major pathway regulating HSC function. Together, these data indicate that ROS-mediated effects on HSC function are reversible. Consistent with this conclusion, catalase, an essential enzyme defensing against ROS stress, ameliorates the loss of HSPC in response to irradiation by reducing IR-induced ROS levels and DNA double-strand breaks (Xiao et al. 2015). Recent study points out that metformin, a medicine to treat type II diabetes, extended mouse life span with certain concentration (0.1% w/w) by reducing oxidative damage accumulation and chronic inflammation (Martin-Montalvo et al. 2013). Furthermore, inhibition of mitochondrial metabolism of HSCs by metformin maintains the integrity of HSCs ex vivo (Liu et al. 2015). Together, inhibition of ROS may be beneficial to maintain stem cell function, especially in the context of stress factors that lead to abnormal high ROS levels. The beneficial effects of antioxidants under physiological conditions, in contrast, remain controversial as hormetic effects that increase health and life span in response to mild stress would also be abrogated by such treatments (Ristow 2014). The heterozygous deletion of *Sod2* resulted in reduced SOD2 protein levels and increased oxidative stress in the context of telomere dysfunction; however, the function of HSC of $Sod2^{+/-}$, $G3mTerc^{-/-}$ was comparable to HSCs of $Sod2^{+/+}$, $G3mTerc^{-/-}$ indicating that ROS elevation does not aggravate the impairment of HSC function in the context of telomere dysfunction (Guachalla et al. 2009). Caloric restriction (CR) is shown to delay aging in many species (Colman et al. 2009; Morck and Pilon 2007), which is partially mediated by reducing ROS (Qiu et al. 2010; Minamiyama et al. 2007). Short-term exposure to CR enhances HSC repopulation potential but impairs lymphoid differentiation capacity partially through insulin-like growth factor 1 and IL-6/IL-7 (Tang et al. 2016). A very recent study indicates that ectopic adipocyte accumulates in aged bone marrow cavities, which limits HSC function through secreting excessive amounts of dipeptidyl peptidase-4, a protease that is a target of diabetes therapies (Ambrosi et al. 2017). However, Lazare et al. reported that

lifelong CR or high-fat diet is not able to affect HSC function (Lazare et al. 2017). Conflicting results may stem from experimental setting, time of CR, or even the composition of foods demonstrated by deficiency of valine which impairs HSC function (Taya et al. 2016). Further study to disclose the effect of duration of CR on HSC functionality or aging would coordinate the difference.

8.5 Protein Homeostasis and HSC Aging

Protein homeostasis (proteostasis), known as synthesis, folding, disaggregation, and degradation, is critical for cellular or organismal functionality, survival, and life span. Proteostasis is tightly controlled by a complex regulatory network, such as autophagy pathway, mTOR signaling, the heat shock factors, the unfolded protein responses, and the sirtuin gene family. Alteration of proteostasis is one of the main risk factors of many diseases, including Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis. Aging correlates with altered proteostasis, and intervention of proteostasis can slow aging and ameliorate disease progression in proteostasis disorders. Decreased protein synthesis is commonly observed with aging, and this may also affect the availability of enzymes that are required for cell maintenance, repair, and metabolic activity. Indeed, age-related changes in the specificity and stability of enzymes have been reported (Labbadia and Morimoto 2015).

There is evidence that a fine-tuned control of protein synthesis is required to maintain HSC integrity (Signer et al. 2014). In addition, protein-degrading mechanisms are essential for HSC maintenance. Along these lines, autophagy, a basic catabolic mechanism of cells, which degrades cellular components through lysosomes, appears to be required to maintain HSC survival during homeostasis and starvation-induced energy deficits. The targeted deletion Atg7, an important gene in autophagy, abolished the long-term reconstitution capacity of HSCs and resulted in a premature aging phenotype under homeostatic conditions (Mortensen et al. 2011). Moreover, Atg12-deficient mice aggravate the sensitivity of HSCs to lose self-renewal in response to starvation, indicating that autophagy protects HSC during severe calorie restriction in vivo (Warr et al. 2013). Although growing data show that decreased autophagy is associated with aging (Rubinsztein et al. 2011), aged HSCs exhibit increased autophagy as marked by elevated numbers of autophagic vacuoles that are absent in HSCs from young mice (Warr et al. 2013). The data suggest that autophagy activation may be indispensable for the survival of HSCs from old mice. The exact mechanism behind this phenomenon needs further investigation.

Mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase that regulates cell survival, protein synthesis, transcription, and autophagy (Kim and Guan 2015; Shimobayashi and Hall 2014). mTOR is composed of two catalytic subunits: mTORC1 and mTORC2. The mTORC1 complex is strongly required for maintenance of HSC function as the deletion of either Raptor or Tsc1 resulted in loss of HSC function (Chen et al. 2008; Magee et al. 2012). Also, the deletion of

Rictor, a component of mTORC2 complex, led to impairments in HSC function albeit less pronounced compared to genetic depletion of mTORC1 complex members (Magee et al. 2012).

In contrast to the negative effects on deleting mTOR complex members, there is evidence that incomplete inhibition of TOR has beneficial effects on stem cell maintenance and the delay of organismal decay during aging. Decreasing TOR activity by dietary restriction (DR) was shown to prolong life span of *S. cerevisiae*, *C. elegans*, and *D. melanogaster* (Powers et al. 2006; Kaeberlein et al. 2005; Jia et al. 2004; Kapahi et al. 2004). DR also prolonged the survival of some mice strains depending on the genetically determined ability to efficiently utilize dietary resources (Rikke et al. 2010). There is evidence that DR and intermittent fasting can improve the functionality of different adult stem cell compartments including skeletal muscle (Ruckh et al. 2012) and HSCs (Chen et al. 2003; Cheng et al. 2014). Interestingly, intermittent fasting also improved the lymphoid output of hematopoiesis in aging mice (Cheng et al. 2014). A recent study showed that the benefits of dietary restriction on delaying organismal aging are mediated by increased hydrogen sulfide (Hine et al. 2015). Dietary restriction stimulates endogenous hydrogen sulfide by repressing mTORC1 and activation of the transsulfuration pathway. Whether increasing the production of endogenous hydrogen will protect HSC and other stem cells from aging-associated decline or DNA damage accumulation remain an interesting question of future research.

There is evidence that mTOR activity increases in aging HSCs and that the inhibition of mTOR activity by rapamycin improves the reconstitution potential of aged HSCs and lymphopoietic output of the aging hematopoietic system. Moreover, these beneficial effects in the hematopoietic system associated with a prolonged life span of aged mice (Chen et al. 2009). Instead, the block of NPRL2, an inhibitor of mTORC1, resulted in defective hematopoiesis (Dutchak et al. 2015). These two studies suggest that inhibition of mTOR activity delays HSC aging, rescues the skewing of HSC, and improves immune function which is induced by the aging-induced skewing in the HSC pool and in myelo-/lymphopoietic output (see above section and Stenderup et al. 2003). Interestingly, there is emerging evidence that mTOR inhibition may also improve immune function in elderly humans (Mannick et al. 2014). However, these studies need to be interpreted with caution as there is experimental evidence that mTOR inhibition can aggravate defects in mitochondrial biogenesis, energy homeostasis, and organ maintenance in the context of telomere dysfunction-induced aging (Missios et al. 2014). It is possible that DR and mTOR inhibition-dependent effects on disease prevention and health span extension are limited to mid-age periods and that advanced aging limits these beneficial effects by impairing the maintenance of energy homeostasis through an increase in energy consumption rates recently reported to occur in senescent cells (Dorr et al. 2013) and telomere dysfunctional tissues (Missios et al. 2014). Such biphasic effects may also explain why DR failed to increase life span in nonhuman primates despite the increases in health parameters (Mattison et al. 2012).

Sirtuins, nicotinamide adenine dinucleotide (NAD)-dependent deacetylases, are a family of enzymes involved in many processes that are important for aging such

as the regulation of metabolism, mitochondrial function, stress responses, and signaling (Lin 2015). In humans, the family consists of seven enzymes, and several human diseases are related with sirtuins (Imai and Guarente 2014). Sirtuins have also been implicated to control the functionality of various types of stem cells in physiological and pathophysiological conditions including skeletal muscle stem cell, HSCs, and LICs (Ryall et al. 2015; Li and Bhatia 2015). The activation of several sirtuin family members including Sirt1 activation in response to DR is implemented in mediating positive effects on several health parameters (Baur et al. 2012; Houtkooper et al. 2012; Giblin et al. 2014). Activation of Sirt1 was also shown to promote hematopoietic stem and progenitor cell expansion and to improve hematopoiesis in mouse models of Fanconi anemia (Zhang et al. 2015). In contrast, inactivation of sirtuins often has negative effects on health. Along these lines, Sirt7 deletion compromises HSCs' function by introducing unfolded protein genes (Hsp10 and Hsp60) in mitochondria (UPSmt), indicating that increases in protein synthesis in response to Sirt7 deletion impair HSC function (Mohrin et al. 2015). Experimental data indicate that HSCs are predisposed to undergo apoptosis in response to unfolded protein response (UPR) activation whereas hematopoietic progenitor cells have the capacity to survive by activating adaptive mechanisms (van Galen et al. 2014). These data indicate that the UPR stress response pathways contribute to maintain a pristine stem cell pool to avoid propagation of protein damages from stem to progenitor cells. These data also fit to the observation that HSCs exhibit lower protein synthesis rates compared to other hematopoietic cells (Signer et al. 2014). Moreover, protein synthesis needs to be tightly regulated in HSCs as both decreases and increases in protein synthesis are detrimental for HSC function. Interestingly, HSCs exhibit an increase in ribosomal biogenesis during aging, indicating that protein synthesis control may be disturbed in this context possibly contributing to aging-associated decline in HSC function. Of note, the deletion of ribosome large 60S subunit paralogs RPL31A and RPL6B prolonged yeast replicative life span (Selman et al. 2009), and inhibition of various genes in the translation initiation complex, including igf-1, eIF4G, and rsks-1, results in life span extension in *Caenorhabditis elegans* (Pan et al. 2007) and mammals (Selman et al. 2009). In HSCs decreases in ribosomal biogenesis were found to promote resistance to endogenous and genotoxic stress (Cai et al. 2015). However, there is also evidence that protein synthesis declines with aging in many organisms (Makrides 1983; Rattan 2010), indicating that effects on manipulating protein synthesis rates may have different outcomes in stem cells and somatic cells and in different stage of the life cycle (see also above discussion on mTOR and DR).

8.6 HSC Aging and Leukemia

Leukemia is a group of hematopoietic cancers and starts from aberrant hematopoietic stem and progenitor cells. Acute myeloid leukemia (AML) is one type of leukemia exhibiting a rapid growth of abnormal white blood cells that accumulate in the bone marrow and interfere with the production of normal blood cells (Fig. 8.1).

AML is the most common myeloid leukemia with poor overall survival (Fernandez et al. 2009). The exact reason of AML is still under debate. But scientists believe that some risk factors promote leukemogenesis, including ionizing radiation, chemicals, aging, and genetic factors. The incidence of AML increases with age: the prevalence increases from 3.8 cases per 100,000 (total average) to 17.9 cases per 100,000 in adults aged 65 years or older. Although significant progress has been made in the treatment of younger adults with AML, the prospects for elderly patients have remained dismal, with median survival times of only a few months (Estey and Dohner 2006; Appelbaum et al. 2006). A significant progress has been made to uncover the molecular mechanism behind AML in recent years. Phenotypic healthy HSCs purified from AML patient in long-term remission after therapy, which carry a set of founder mutation in certain genes and acquire more driver mutations sequentially to finally transform into AML by further clonal evolution and the acquisition of FLT3-ITD (Jan et al. 2012). Of note, clonal hematopoiesis increases with aging in hematopoietic cells of healthy humans (Xie et al. 2014; Jaiswal et al. 2014; Genovese et al. 2014; McKerrell et al. 2015). Mutant clones are rarely detectable in peripheral blood of young individuals (<40 years), but this frequency increases exponentially with age. The majority of mutations locate in a few genes: DNMT3A (it is by far the most frequently affected gene), followed by TET2, ASXL1, JAK2, TP53, GNAS, PPM1D, BCORL1, SF3B1, and SRSF2. The presence of somatic mutation in peripheral blood represents a risk factor for the prospective development of hematologic malignancies, other aging-associated diseases (such as coronary heart disease and ischemic stroke), and overall survival in the elderly (Jaiswal et al. 2014). Together, these studies indicate that the clonal dominance of mutations in specific genes is highly selected in aging HSCs and/or progenitor cells. While mechanism that drive clonal expansion of mutant stem and progenitor cells in aging remain largely elusive at this stage, it is evident that these mutations provide a ground stage for the development of diseases including leukemia. Consistent with this interpretation, DNMT3A mutation-bearing HSCs behave as preleukemic HSCs by showing multilineage repopulation advantage over non-mutated HSCs and better tolerance against chemotherapy (Shlush et al. 2014). The discovery of clonal dominance of mutant stem and progenitor cells as pre-disease conditions in aging holds the promise that the targeting mutant stem and progenitor cell clones could represent a novel therapeutic approach to prevent aging-induced diseases and cancer. Several promising attempts in this direction have already been reported.

8.6.1 Vaccination to Target Mutation-Bearing Cancer Cells

Isocitrate dehydrogenase type 1 (IDH1) is recurrently mutated in AML patients (Mardis et al. 2009). Most of the affected patients carry IDH1 R132 mutations that strongly reduce the catalytic activity of the IDH1 enzyme. Interestingly, vaccination with the mutant peptide was shown to induce antitumor immune responses in humanized mouse models (Schumacher et al. 2014). As a caveat, the reduced

vaccine efficiency as a consequence of aging-induced impairments in immune function will likely limit such approaches (Haq and JE 2014).

8.6.2 Small Molecules to Target Mutation-Bearing Cancer Cells

IDH2 mutations result in a specific hypermethylation signature, which impairs hematopoietic differentiation and predisposes HSC to preleukemic stem cells of acute myeloid leukemia (AML) (Figueroa et al. 2010). A small molecule, AGI-6780, selectively inhibits IDH2 R140Q-bearing tumor cells and induces differentiation of TF-1 erythroleukemia and primary human acute myelogenous leukemia cells in vitro (Wang et al. 2013). Treatment of AGI-6780 reverses the histone and DNA hypermethylation pattern within weeks and alteration of specific genes induced by IDH2 R140Q mutation (Kernytsky et al. 2015). IDH1 is frequently mutated in various cancers (Mardis et al. 2009; Figueroa et al. 2010; Turcan et al. 2012). A selective IDH1 R132H inhibitor (AGI-5198) blocked the production of R-2-hydroxyglutarate (R-2HG), which is sufficient to promote leukemogenesis (Losman et al. 2013; Rohle et al. 2013). Together, these studies raised the potential therapeutic role of small molecule in treating specific mutation-bearing tumor cells. Recently, massively parallel DNA sequencing of clinical cancer samples revealed that *DNMT3A* is one of the most frequently mutated genes across a number of hematological malignancies. Both young and old AML patients with *DNMT3A* mutations exhibited inferior outcome compared with non-*DNMT3A* mutation AML patients (Ley et al. 2010). Furthermore, both Dnmt3a-deficient and Dnmt3a R878H mutation mice resulted in expansion of HSPC. The balance between self-renewal and differentiation was disrupted by the loss of Dnmt3a (Challen et al. 2011; Dai et al. 2017). AML patients carrying DNMT3A R882H also exhibited resistance to daunorubicin-based chemotherapy with a possible mechanism of impaired nucleosome remodeling (Guryanova et al. 2016). 5-Azacytidine and rapamycin showed a potential treatment effect for both $Dnmt3a^{R878H/WT}$ and human DNMT3A-mutated leukemic cells (Dai et al. 2017; Xu et al. 2014). Recent study suggested histone H3K79 methyltransferase DOT1L is a promising therapeutic target for the treatment of DNMT3A-mutant AML. DOT1L inhibitors, EPZ5676 and SGC0946, can reduce primary DNMT3A-mutant AML samples' colony-forming capacity (CFC) and induce terminal differentiation ex vivo and reverse $DNMT3A^{R882H}$-mediated aberrant transactivation of HSC pluripotency-related genes (Rau et al. 2016; Lu et al. 2016). However, none of the above molecules can target a specific mutation, such as the most hotspot mutation R882, which may cause undesirable side effects. Currently, our group identified a small molecule via high-throughput screening which exhibited high affinity to DNMT3A R882H while low affinity to wild-type DNMT3A which is promising to develop into a molecular targeting drug.

8.7 Perspective

Multiple processes contribute to the aging-induced decline of HSC function leading to defects in blood regeneration, immunosenescence, and myeloproliferative diseases. Accordingly, dysfunction of HSC during aging contributes to aging-related disorder in the blood system including leukemia. Emerging topics in research on stem cell aging largely focus on molecular mechanisms that drive aging-induced defects in HSC function and the clonal dominance of mutant stem and progenitor cell clones. The understanding of these processes is beneficial to develop future therapies and intervention strategies that have the potential to prolong the functionality of stem cells during aging, thus improving health and quality of life in the elderly.

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Microbiota and Aging

9

Maoyang Lu and Zhao Wang

Abstract

The human gut microbiota is a huge ecosystem that provides lots of functions for host development, immune system, and metabolism. Gut microbiota is linked to lots of diseases, including human metabolic diseases such as obesity, type 2 diabetes (T2D), irritable bowel syndrome, and cardiovascular disease (CVD). Few studies, however, have noted the relationship between aging and microbiota; the connection between aging and microbiota remains largely to be researched. In this review, recent research findings are summarized on the role of gut microbiota in aging processes with emphasis on therapeutic potential of microbiome-targeted interventions in antiaging medicine.

Keywords

Gut microbiota · Aging · Fecal transplantation · Aging-related diseases

9.1 Introduction

The human gut microbiota is a huge ecosystem that provides lots of functions for host development, immune system, and metabolism. Gut microbiota is linked to lots of diseases, including human metabolic diseases such as obesity, type 2 diabetes

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(T2D), irritable bowel syndrome, and cardiovascular disease (CVD). Few studies, however, have noted the molecular mechanisms; the connection between host and microbiome remains largely to be researched. We review how aging may affect gut microbiota in the complex interactions of diet, microbiota, and host metabolism and show the new theories about the signaling pathway of modulating gut microbiota.

Human intestinal tract has a large number of bacteria; these bacteria are related to the host immune system development, metabolic regulation, nutrient digestion and absorption (Sommer and Backhed 2013). Because of the application of system biology approaches and new computational tools, there has been an increasing interest in exploring the gut microbiota and their combined genomes, the microbiome, as diagnostic and therapeutic targets for prolonging human lifespan and treating aging-related diseases (Olle 2013). Through plenty of experiments, the molecular mechanism of gut-host interaction has been elucidated.

Microbiota is defined as the collection of microbial taxa in a given environment. The concept of microbiome is the collection of the genes/genomes encoded by the microbiota. Compositional and functional changes of the human gut microbiome have been linked to lots of chronic metabolic disease, like malnutrition (Subramanian et al. 2014) as well as obesity (Turnbaugh et al. 2009) and obesity-associated diseases such as cirrhosis (Qin et al. 2014). Then, alterations of the gut microbiome have also been linked to intestine-related diseases, including inflammatory bowel disease (IBD) (Palm et al. 2013), colorectal cancer (Zackular et al. 2013), and neurodevelopmental disorders (Hagemann et al. 2013). Changes in lifestyle and diet have been argued to contribute to the shifting gut microbiota ecology.

Obesity, T2D, and IBD are characterized by reduced fecal microbial diversity, and studies have shown that uses of dietary emulsifier alter the gut microbiota's composition, which results in intestinal inflammation, devastation of gut barrier, and development of metabolic syndrome. These findings suggest that the modern lifestyle, especially diet, has the potential to affect the gut microbiota, which may contribute to disease development. Therefore, understanding factors which influence the gut microbiota might lead to the finding of new therapies for both metabolic and inflammatory diseases.

There are three major factors which influence the composition of gut microbiota, including host genetic background, diet, and microbes. Human genetics might play a role in shaping the composition of the gut microbiota. For instance, people homozygous for loss-of-function alleles of the FUT2 gene have an altered microbiota. FUT2 encodes an enzyme which is required for the fucosylation of surface carbohydrates on intestine mucosal linings. Loss in FUT2 altered both the composition and the function of the gut microbiota. FUT2 gene has been linked to Crohn's disease and IBD, suggesting that an altered gut microbiota may explain the association between FUT2 gene background and increased possibilities to Crohn's disease. Besides FUT2 gene, FXR gene and NOD2 gene also play a vital role in shaping gut microbiota. However, further investigations are needed to determine the extent to which host genotype impacts and shapes the microbiome, as studies in mice show that environmental factors like diets might have nominating effects (Goodrich et al. 2014). Recently, there have been new reports on the relationship between host genetic background and gut microbiota, caspase recruitment domain family

member 9 (CARD9), a susceptibility gene for inflammatory bowel disease (IBD) that functions in the immune response against microorganisms, which promotes recovery from colitis by promoting interleukin (IL)-22 production, and Card9$^{-/-}$ mice are more susceptible to colitis. Card9 can change the composition of gut microbiota, when transplantation the card9-/- mouse's feces to normal mice, it can lead to the occurrence of colitis in mice (Lamas et al. 2016). This research proves that there is exactly relationship between host genetic background and composition/function of the gut microbiota, demonstrating the mechanism by researching the production of microbial metabolites.

Energy intake and the nutrient composition of the diet affect human health and impact the composition of the human gut microbiota; in particular, recent studies in mouse performed on different genetic background and different living environment show that diet has a stronger effect than host genotype in determining gut microbial composition (Zang et al. 2010). The gut microbiota responds to dietary interventions very quickly, and short-term consumption of diets composing of either animal or plant products can alter the overall structure of the gut microbiota (Zhang et al. 2012). For example, for people with proteins and fats as the main food, *Bacteroides* dominated the gut microbiota, while for those with fiber and carbohydrate as the main foods, the gut microbiota is dominated by the genus *Prevotella* (Wu and Chen et al. 2011).

The microbes, including pathogens and prebiotics, are an important key of the way of shaping gut microbiota. Recent research shows that the interaction between microorganisms and microorganisms also affects the gut microbiota. For example, *Salmonella* and *Clostridium difficile*, two antibiotic-associated pathogens, improve their numbers and activities after antibiotic treatment by utilizing microbiota-liberated mucosal carbohydrates such as sialic acid (Ng et al. 2013). Now, as beneficial bacteria, probiotics should be mentioned. Probiotics is a living microorganisms that confer a health benefit on the host. Prebiotics is an activator of probiotics. Prebiotics is dietary ingredients that are fermented by specific gut microbes, which results in specific changes in the composition of the gastrointestinal tract microbiota, making benefits on host health (Hoffmann et al. 2013).

9.2 The Gut Microbiota's Changes During Aging Process

As mentioned above, the body's aging process is accompanied by the occurrence and development of inflammation; meanwhile the function of each organ has declined. Conversely, gut microbiota may have their own unique way of changing (O'Toole and Jeffery 2015). At the same time, it cannot be ignored that the elderly usually have a variety of comorbidities, changes in diet and exercise habits, and other changes associated with gut bacteria that affect the gut microbiota. Therefore, the question of how to interact with gut bacteria still deserves further exploration.

Compared to evidence related to aging and inflammation, less is known regarding associations between aging and the microbiome. In fact, in contrast to the thousands of peer-reviewed publications on aging and inflammation, a PubMed search for "aging and microbiome" yielded only 466 results, and a search for "aging and dysbiosis" yielded a mere 34. Moreover, only a handful of studies to date have

investigated the aging microbiome in humans. Still, at least two early studies in this area have documented that advanced age is associated with changes to both the composition and stability of gut microbiota (Biagi et al. 2010). Biagi et al. reported that a group of centenarian from Northern Italy displayed low species diversity compared to younger adults (~30 years of age). They also noted specific changes within *Firmicutes* (one of the two dominant phyla commonly found in the gut) subgroups and enrichment of *Proteobacteria*—a group containing many opportunistic bacteria which can overtake commensal bacteria and induce pathology. These microbiome changes were also characterized by a loss of genes for short-chain fatty acid production and an overall decrease in the saccharolytic potential, while proteolytic functions were more abundant than in the intestinal metagenome of younger adults (Rampelli et al. 2013). Interestingly, these changes in bacterial content were also moderately associated with circulating plasma concentrations of inflammatory cytokines interleukins six (IL-6) and eight (IL-8). Surprisingly, however, despite these interesting findings is happened among the centenarians, we did not find significant differences in microbiota composition between the younger adults and a group of older adults with an average age of 70 years. In contrast, a study of gut microbiora in Ireland found that core populations of people over the age of 65 did indeed change. These changes are mainly manifested in the substantial increase in the proportion of Bac- teroides spp. and Clostridium compared to younger individuals. Gut microbiota's diversity of the elderly will decline, mainly in the diversity of related species, including Prevotella , which may lead to the instability of the composition of the entire microbial community. However, it would not be ignored that the difference in gut microbiota between the elderly is so great that the prediction of the phenotype is more difficult to carry out. A few key factors are the predominant predictors of gut microbiota in community-dwelling and long-term care residents, such as diet and the use of antibiotics. Differences in cohort studies in Italy and Ireland may be explained by diet differences.

The composition of gut microbiota can change significantly with aging and aging-related diseases (Lakshminarayanan et al. 2014). Age-related changes in gut physiology, such as gastric motility disorders, achlorhydria, and degenerative changes in the enteric nervous system, have a significant impact on the composition and function of gut microbes (Konturek et al. 2015). Long-term stimulation of the immune system can lead to decreased immune system function, leading to immunosenescence, which in turn causes the above age-related differences. Subsequent to this are many aging-related diseases, including gastrointestinal-related (*Clostridium difficile* colitis) and other (cachexia, frailty, cancer) (Bischoff 2016) diseases. Such inflammatory state might make the host more sensitive to gut bacteria.

The age-related changes in the gut microbiota composition include a decline in microbiota diversity, a decrease in saccharolytic bacteria and an increase in proteolytic bacteria, a decreased abundance of core (dominant) species and an increased abundance of subdominant species, an increase of certain *Proteobacteria*, a reduction of bifidobacteria, and also a decrease of the ratio of *Firmicutes* to *Bacteroides* (F/B) (Perez et al. 2014). Taking the number of bifidobacteria as an example, the

number of bacteria will decrease from 90% of the total number of colonic microbial communities when as a baby to 5% as an adult. With the aging, this bacteria will decrease rapidly in centenarians (Riviere et al. 2016).The pronounced changes in gut microbiota occur through the transition from adulthood to old age. Among the elderly, there is a decrease in the diversity of gut microbiota and a greater variation in the differences between individuals than young people, with reduced numbers of bifidobacteria, *Firmicutes*, *Faecalibacterium prausnitzii*, *Clostridium* cluster XIV, and *Blautia coccoides-Eubacterium* rectal and greater presence of *Bacteroidetes* and *Enterobacteriaceae* (Rondanelli et al. 2015). The centenarian's microbiota has shown to be less diverse than in adult persons and has demonstrated decreased levels of *Bifidobacterium*, *Bacteroides*, and *Enterobacteriaceae* and increased *Clostridium* (Biagi et al. 2010). Such aging-associated differences in gut microbiota cannot necessarily be caused by aging; this change is mainly due to the decline in body health, including malnutrition, the use of antibiotics, etc. Therefore, recent studies have suggested that the loss of the core community of intestinal gut microbiota is more related to aging-associated frailty than with chronological age. With the aging of the body, the body will have a series of aging-related diseases, such as chronic inflammation, neurodegeneration, cognitive decline, frailty, and type 1 and type 2 diabetes. Age-related changes in gut microbes are a very important factor in these disease states.

9.3 The Role of Microbiota During Aging Process

The available data indicate that the composition of the intestinal flora can affect the rate of aging. The age-dependent relationship between host and gut microbiota is influenced by many factors, such as age-related lifestyle changes, inflammation, and the hobby (Candela et al. 2014).

Evidence suggests that changes in gut flora predict the lifespan of humans. Studies have shown that the composition of young people and 70-year-olds is relatively similar, but the difference between centenarians is very quite obvious. Among centenarians, the major changes in gut flora are characterized by the reconstruction of *Firmicutes* population and enrichment in *Proteobacteria*. This phenomenon is mainly associated with an increase in the inflammatory status of the centenarians, such as an increase in a range of inflammatory markers. The changes in the centenarian microbiome are accompanied by a decrease in *Faecalibacterium prausnitzii*, which is reported to have strong anti-inflammatory activity. In addition, *Eubacterium limosum* is considered to be a landmark in predicting longevity because of its substantial increase in the population of longevity elders. In the recent analysis of the super-long-lived elderly, the levels of several probiotics have risen dramatically (e.g., *Akkermansia*, *Bifidobacterium*, and *Christensenellaceae*). In addition, from the point of bacterial function, the main functional characteristics of the dominant microflora of centenarians are the decrease of short-chain fatty acid synthesis and the decrease of glycolysis ability. On the contrary, the proteolytic function is

significantly stronger than young people (Rampelli et al. 2013). In this study, 116 microorganisms were identified as markers of longevity.

Targets for antiaging drugs are mainly bifidobacteria and butyrate-producing colon bacteria, such as *Faecalibacterium prausnitzii* and *Roseburia*. The short-chain fatty acid butyrate is the product of bacterial metabolism in the colon. It is a source of colonic epithelial cells and helps to maintain the integrity of the intestinal barrier and improve anti-inflammatory and anticancer abilities. It can also exert its beneficial metabolic effects via prevention of metabolic endotoxemia, enhanced mitochondrial activity, and activation of gut gluconeogenesis (Hartstra et al. 2015). Moreover, butyrate has many other functions, such as preventing metabolic endotoxemia and enhancing mitochondrial activity, and it also regulates epigenetic processes by inhibiting histone deacetylase activity. Therefore, butyrate has strong therapeutic potential in age-related diseases.

Intestinal microorganisms can cause age-related inflammation and premature death in mice. Dysbiosis of gut microbes in older mice may result in intestinal leakage that releases the bacterial products which causes inflammation in the body, thereby impairing immune function and reducing lifespan. In fact, a similar phenomenon has been found in the human body. Often, older adults with high levels of inflammatory factors in the body are more likely to have frailty, reduced self-care ability, and hospitalization. At the same time, they are also more vulnerable to infections, dementia, and cardiovascular diseases. However, so far, it is unclear that how the composition of gut microbes, inflammation and deterioration of the health of the elderly are related. Aging related inflammation leads to macrophage dysfunction and tissue damage. They feed a group of mice under germ-free conditions and compared it to that of a conventionally cultured control mouse. Compared with the conventional cultured mice, the germ-free mice showed no aging-related increase in inflammation and have longer average life expectancy. If mixed with older mice, more of germ-free mice have aging-related inflammation; these are not the effects when mixing with young mice. In normal humans and mice, the aging process is accompanied by an increase in the levels of proinflammatory cytokines such as tumor necrosis factor (TNF) and interleukin 6 (IL-6) in the blood and tissues. Among them, the increase of TNF will damage the function of macrophages, and increase the permeability of the intestinal wall. In contrast, GF mice do not show an increase in TNF levels with age. Consistent with this, TNF-deficient mice do not develop age-related inflammation. In normal mice, Humira, an anti-TNF monoclonal drug, can reduce the harmful changes in the intestinal microbiota due to aging process. This shows that the composition of the gut microbiota may vary depending on the host's inflammatory status. In general, leakage of intestinal flora toward the intestinal wall occurs throughout life. However, with age, this phenomenon induces an inflammatory response that can further cause an ecological imbalance in the gut microbiota. An imbalance in the gut microbiota increases the permeability of the gut, thereby increasing the leakage of intestinal flora. This positive feedback process will increase with aging (Thevaranjan et al. 2017) (Fig. 9.1).

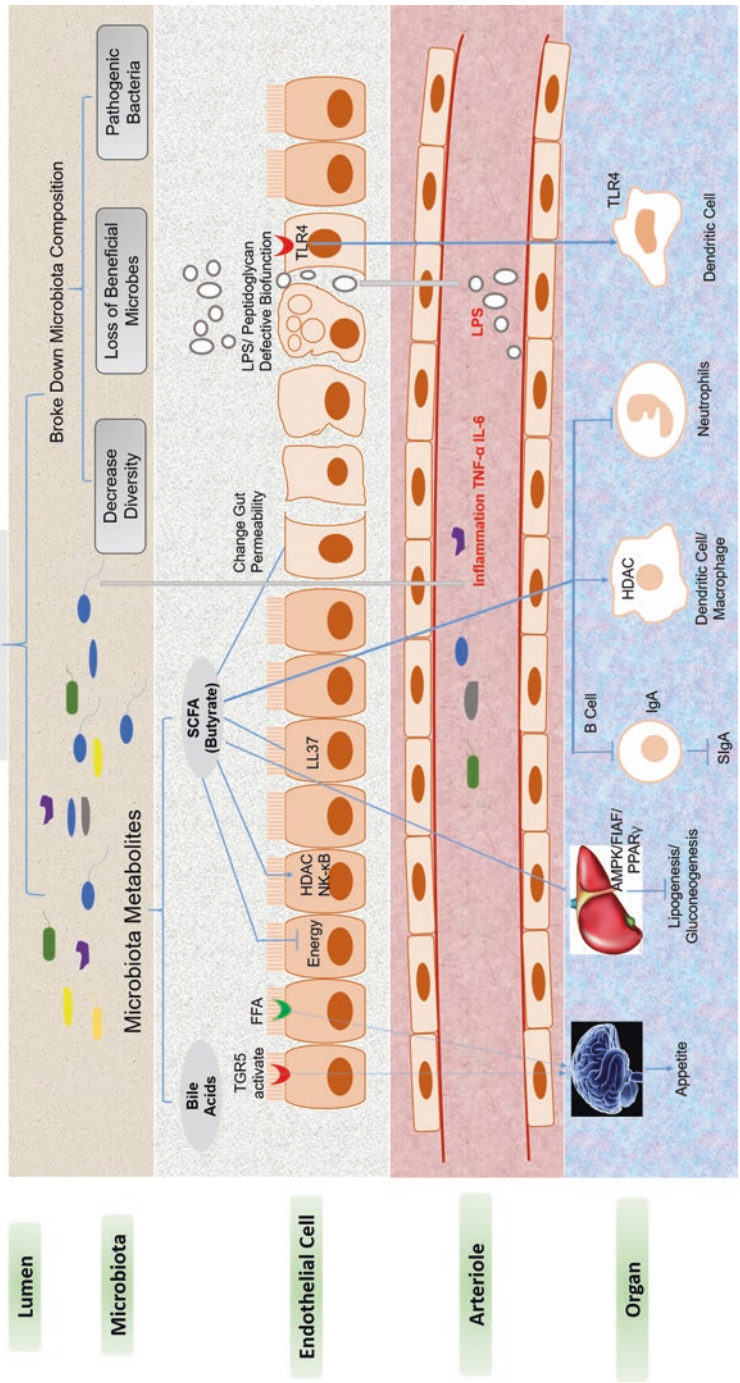

Fig. 9.1 The molecule signaling pathway between gut microbiota and aging
During the aging process, the metabolite and composition of gut microbiota are changed which caused the leakage of gut and systematic inflammation

9.4 The Gut Microbiota and Aging-Related Diseases

With aging process, the body will have a series of diseases, such as Alzheimer disease and osteoporosis and so on. The gut microbiota will change during this diseases process. We will show how aging-related diseases interact with gut microbiota in this sector.

9.4.1 Alzheimer Disease

At present, the mechanism of gut microbiota participating in AD has been tentatively explained. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the human central nervous system. When patients have disorders of the gut microbiota, especially when the number of bifidobacteria and *Lactobacillus* is reduced, GABA levels in the intestine will be affected, further leading to a decrease in GABA levels in the central nervous system. Glutamate is the major excitatory neurotransmitter in the human central nervous system. N-methyl-D-aspartate glutamate receptor (NMDA) receptor is an important glutamine acid receptor, mainly involved in the regulation of neuronal survival, dendrites and axonal structures, and synaptic plasticity (Hu et al. 2016). Neufeld et al. (Matsumoto and Benno 2004) reported that the expression of NMDA receptor NR2B mRNA in the hippocampus of germ-free mice is significantly downregulated, indicating that gut microbiota is associated with the expression of NMDA receptors. The above study suggests that gut microbiota may participate in the pathological process of AD by affecting metabolism.

Amyloid is an aggregate of insoluble proteins formed by misfolding and plays an important role in the pathogenesis and progression of AD. A variety of gut bacteria, such as *Bacillus*, *Staphylococcus aureus*, *Streptomyces*, and *E. coli*, can secrete amyloid protein or its degradation products; it can induce oxidative stress response, further activate microglia, and release inflammatory factors, such as TNF-α, IL-1, IL-6, etc. As the aging process, the permeability of the intestinal epithelial layer and the blood-brain barrier increases, and these products can enter the brain through the blood-brain barrier and further induce the occurrence of AD. Clinical studies have reported that bifidobacteria, lactic acid bacteria, can regulate the gut microbiota, improving the cognitive ability of AD patients. The mechanism may be related to the exogenous polyamines produced by gut microbiota. Polyamines not only inhibit the production of inflammatory cytokines, but also have antioxidant effects. Intestinal microorganisms can regulate the metabolism of tryptophan, further affecting the content of 5-HT in vivo. The gut microbiota's metabolites can promote the secretion of serotonin by enteroendocrine cells, thereby regulating the balance of AD neurotransmitter. In addition, probiotics can also promote the production of neurotrophic factors in the brain, reduce the activity of inflammatory cytokines, thereby contributing to the prevention and early treatment of diseases associated with cognitive disorders.

In conclusion, the pathogenesis of AD is closely related to the imbalance of gut microflora. Transplantation of probiotics can regulate the gut microbiota which can activate the immune system, increase the release of neurotransmitters in the brain, and effectively improve AD gastrointestinal and neurological symptoms. Therefore, an in-depth study of the mechanism of intestinal microbial involvement in AD will provide a new target for the treatment of AD.

9.4.2 Osteoporosis

In recent years, research on gut microbiota and osteoporosis has become a hot topic in orthopedic research. The McCabe study (McCabe et al. 2015) points out that gut microbiota can affect the bone metabolism through the release of small molecules such as estrogen and serotonin and immune regulation and affect absorption and metabolism of calcium and phosphorus. Sjögren (Sjogren et al. 2012) finds that mice with a lack of gut microbiota have approximately 40% higher bone density in the distal femur than normal mice. In addition, the expression level of osteolytic cytokines such as IL-6 and TNF-α in bones of germ-free mice is significantly reduced. Therefore, the reduction of osteoclasts in germ-free mice may also be caused through regulation of the immune system. The number of osteoclasts in sterile mice is relatively low, and the formation of T cells and osteoclasts is significantly reduced, but the rate of bone formation is not reduced through culture of bone marrow mesenchymal stem cells (BMSCs). They also point out that the expression of inflammatory factors in germ-free mice is relatively small, but there is no difference in serum calcium and serum phosphorus levels. In conclusion, the increase in bone mass in germ-free mice is due to the lack of bacterial as antigen to cause immune response.

Although animal models have confirmed that gut microbiota increases inflammation and accelerates postmenopausal bone resorption, no studies have yet confirmed the relationship between human gut microbiota and osteoporosis, suggesting that bone mass occurrence is the result of many factors. However, we have reasons to believe that disorder in the gut microbiota is one of the key factors leading to osteoporosis.

9.5 Regulation of Gut Microbiota and Health

At present, there are several methods for prolonging lifespan: calorie restriction, drugs, food, and gene regulation. However, these methods are mainly based on the conclusions obtained from experimental animals, and there is no real clinical trial. These methods of prolonging life will change the body's health and regulate the gut microbiota. So we will review the interaction between the gut microbiota and anti-aging methods.

9.5.1 Caloric Restriction

In various experiments for promoting health and longevity, changes in gut microbiota have been reported. Alterations in the composition of the gut microbiota are revealed in various healthspan-promoting interventions. In a number of studies, an association has been found between the intestinal microbiota composition and weight loss caused by CR, a most reproducible life-extending strategy now. The F/B ratio, in particular, is consistently found to be increased in obesity and reduced with weight-loss-producing CR-based interventions. For example, in the study of the surgical and dietary weight-loss therapy for obesity, the energy-reabsorbing potential of the gut microbiota, indicated by the F/B ratio, is decreased by CR and increased following laparoscopic sleeve gastrectomy (Damms-Machado et al. 2015). Remarkably, the *Firmicutes* changes were accompanied by alterations in butyrate-producing bacterial species in both groups. The F/B ratio is also significantly decreased in obese individuals receiving a weight-loss dietary intervention (Remely et al. 2015). The weight gain-causing bacteria can, in turn, induce the expression of genes linked to carbohydrate and lipid metabolism thereby influencing dietary energy harvest. In animal models, the CR-induced life extension is accompanied by structural modulation of gut microbiota. For example, calorie restriction throughout the life process can significantly alter the gut microbiota structure in mice. Calorie restriction can significantly upregulate strains that are positively correlated with longevity, e.g., the genus *Lactobacillus*, and downregulate phylotypes negatively associated with lifespan (Zhang et al. 2013). Such CR-induced changes were accompanied by significantly reduced levels of serum lipopolysaccharide-binding protein, suggesting that a structurally balanced architecture of gut microbiota may be established via CR. The authors suggest that CR can cause health benefits for the host through reduction of antigen load from the gut. Recently, it is confirmed that the gut microbiota will be predominant by the bacterium *Lactobacillus* only after 2 weeks of fasting and the intestinal bacteria-derived antigen in the blood of the mice could be significantly reduced and the level of systemic inflammation could also be reduced. The researchers isolate the predominant strain of *Lactobacillus lyrhei* from the intestinal tract of fasting mice and found that this strain can reduce the production of inflammatory factors in the Caco-2 cell model in vitro and extend the lifespan of nematodes. Not only that, in germ-free mice transplanted with old mouse's gut microbiota, this strain of bacteria can significantly improve the intestinal barrier damage caused by old flora, thereby reducing the intestinal bacteria-derived antigens in the blood and reducing the age-related systematic inflammation (Pan et al. 2018).

9.5.2 Genetic Modulation

Nematodes and drosophila have become relatively good model organisms for studying aging due to their short life cycles. The researchers identified 29 bacterial mutants in *E. coli* that could significantly extend the life of the host by 10–40%. One

research is about nematodes. The results showed that about half of these 29 longevity-promoting bacteria are capable of drastically reducing the damage caused by tumor expansion and A-β protein deposition. This means that these bacterial mutants can not only prolong life but also significantly improve quality of life. At the same time, these bacterial genes associated with the longevity of the host have been shown to be involved in a variety of signaling pathways that regulate aging, such as the insulin signaling pathway, the mTOR signaling pathway, and the caloric restriction. This shows that bacteria can affect the lifespan of the host through a variety of molecular signaling pathway (Guo et al. 2014). Another research is about drosophila. The amount of bacteria in the intestine of *Drosophila* increases significantly with age, leading to an inflammatory state. This imbalance is driven by long-term activation of the stress response gene FOXO, which inhibits the activity of a class of molecules called PGRP-SCs (homologues of human PGLYRPs). PGRP-SCs regulate the body's immune response to bacteria. Inhibition of PGRP-SC results in the deregulation of a signaling molecule, NFkB, that plays an important role in initiating an effective immune response to enteric bacteria. The resulting immune imbalance leads to the proliferation of bacteria, triggering an inflammatory response and generating free radicals. In the intestine, free radicals cause excessive proliferation of stem cells, leading to abnormal epithelial hyperplasia. When researchers increased PGRP-SC expression in intestinal epithelial cells, the bacterial balance is repaired, and stem cell proliferation is limited. Simply enhancing the function of PGRP-SC is sufficient to prolong the lifespan of drosophila (Han et al. 2017).

9.5.3 Food

Gut microbiota is the main participant in food digestion and is involved in the separation, synthesis, and absorption of major nutrients such as carbohydrates, fats, proteins, and vitamins. Both low- and high-sugar diets affect the structure of the gut microbiota. A low-sugar diet will alter the microbial structure of the intestine, reduce the number of intestinal tumors in the mutant mice, and at the same time reduce the levels of butyrate-producing microorganisms (Belcheva et al. 2014), whereas a high-fat/high-sugar diet will alter the intestinal microflora structure of mice and promote mice developing obesity (Parks et al. 2013). In addition, the proportion of dietary fiber in the diet significantly affects the microbial structure of the intestine. A high-fiber diet increases the number of *Bifidobacterium* in the intestine, and a low-fiber diet increases the number of *Bacteroides* and *Prevotella* (Connolly et al. 2010). The products of carbohydrate fermentation by intestinal microbes are mainly SCFAs, including acetic acid, butyric acid, and propionic acid. Compared with people with protein and fat as their main dietary constituents, the content of short-chain fatty acids in intestinal microbial metabolites is relatively high among people who have long-term carbohydrates as their main diet. Gut microbiota participates in the metabolism of body proteins. On the one hand, it provides a nitrogen source for its own growth. It also helps the host to decompose and synthesize

essential amino acids to meet physiological needs. Lactoferrin is a natural glycoprotein that is mainly found in breast milk and has a variety of biological functions. It can regulate the growth and reproduction of iron-containing bacteria by recovering soluble iron from intestinal fluids. The sIgA in breast milk plays an important role in agglutinating pathogenic bacteria and neutralizing viral toxins, which can prevent neonatal intestinal infections (van der Wielen et al. 2017). It has been reported that certain fruits can prolong life by regulating intestinal flora, such as pomegranate. When the gut microbiota transforms the urolithin A in the pomegranate, they can prompt the body's muscle cells to protect themselves from aging (Ryu et al. 2016).

9.5.4 Drugs

At present, from the experiments of nematodes, drosophila, and mice, metformin and rapamycin drugs can significantly prolong the life of the organism. Metformin is mainly used for the treatment of diabetes. In addition to diabetes, metformin has also begun to be used for diseases, such as cancer, cardiovascular diseases, Alzheimer disease, obesity, retinopathy and other diabetic syndromes, nephropathy, etc., and is known as the "magic drug." Metformin use significantly increased the ability to produce butyrate and propionate in the intestinal flora. Metformin significantly changes the bacterial composition of diabetic patients. Metformin will significantly increase the proportion of the bacterial genus *Sutterella*. Metformin also increased the catabolism of many amino acids including glycine and tryptophan. From a molecular mechanism perspective, metformin can regulate glucose synergistic protein-1 (SGLT1) in the upper small intestine, activate SCLT1-dependent signaling pathways, and reduce glucose production (Bauer et al. 2018). Surprisingly, as another antiaging drug, it prevents the expansion of intestinal stem cells (Igarashi and Guarente 2016). More research is needed to focus on the results of antiaging drugs in humans in the future.

9.6 How Microbiota Regulates Lifespan and Aging

If you would like to check the relationship between gut microbiota and lifespan, examining axenic culture process is a good way. When having sterile culture, the lifespan of *C. elegans* has been increasing twofold (Houthoofd et al. 2002). As we all know, caloric restriction can prolong lifespan. Obviously, bacteria are food source; no one can exclude if dietary restriction is beneficial for extending lifespan. There are different researches checking how sterile culture influences drosophila lifespan and gets different results. There are differences between sex and axenic culture; the results are that axenic culture can shorten males' lifespan while not female (Brummel et al. 2004). On the contrary, Ren, Tower, and colleagues report that NO bacteria have no influence on males' lifespan (Ren et al. 2007). Recently, Clark reported sterile culture can extend females' lifespan, while Petkau reported that antibiotic which can break down the drosophila gut microbiota can also extend

fly lifespan. Beside lifespan, intestinal aging has been delayed through antibiotic treatment. Clark attempted to transplant the stool homogenates of aged drosophila to young drosophila and the structure of the stool changes dramatically and the lifespan of drosophila decreased. Taken together, these experiments demonstrate that the gut flora is closely related to the lifespan and mortality of *Drosophila* (Clark et al. 2015).

In addition to aseptic culture, there are other cases to study the relationship between bacteria and lifespan. Regulation of *C. elegans* and *E. coli* in intestinal tract content can regulate nematode lifespan. For instance, kanamycin administration can delay the proliferation of *E. coli* in the *C. elegans* intestine and increase the lifespan of *C. elegans*. However, some reports show that with the aging process, the accumulation of bacteria in different individuals is quite different. Many *C. elegans* do not accumulate bacteria (Virk et al. 2016). This shows that bacterial accumulation is not the cause of age-related disease. Therefore, different *C. elegans* may die for different reasons. All in all, although there are individual differences, it has been recognized that bacterial pathogenicity can promote *C. elegans* death.

At present, the molecular mechanism of how *C. elegans–E. coli* system affects the lifespan of the host through the intestinal flora has already begun to be studied. This model is relatively simple and allows for easy screening of *E. coli* mutants. Recent studies have identified certain mutant strains that extend lifespan and have discovered the molecular mechanisms how gut microbiota regulates the lifespan of the host. *E. coli* mutants prolong the lifespan of the host by inhibiting the production of folic acid (Virk et al. 2016). A number of additional studies have demonstrated that diffusible molecules originating in bacteria can impact *C. elegans* lifespan (Heintz and Mair 2014). *C. elegans* lack the enzyme nitric oxide (NO) synthase. The source of NO is from bacteria. It has been reported in the literature that NO produced by bacteria can prolong *C. elegans* life (Gusarov et al. 2013). Another mechanism is that small endogenous noncoding RNA expressed by *E. coli* plays an important role in regulating the lifespan of *C. elegans*, and the main function is lncRNA DsrA (Liu et al. 2012). Therefore, bacterial-derived molecules can regulate *C. elegans* lifespan.

9.7 Conclusion and Expectation

Over the past decades, researchers have established a link between the alteration of gut microbial composition and aging and aging-related disease. Aging affects the host health status by modulating the composition of the gut microbiota. It has been found that gut microbiota changes during aging period. In this review, recent research findings are summarized on the role of gut microbiota in aging processes with emphasis on therapeutic potential of microbiome-targeted interventions in antiaging medicine.

In the future, new tools and new approaches are needed for further investigations to find how to prolong lifespan and treat aging-related diseases.

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The paragraph "Compared to evidence related to aging … with an average age of 70 years" in Sect. 9.2 of this chapter was re-used from the original work "Buford, T.W. (Dis) Trust your gut: the gut microbiome in age-related inflammation, health, and disease. Microbiome 5, 80 (2017) doi:https://doi.org/10.1186/s40168-017-0296-0".

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Intestinal Homeostasis and Longevity: *Drosophila* Gut Feeling

10

Xiaolan Fan, Uma Gaur, and Mingyao Yang

Abstract

The association between intestinal homeostasis and life span has caught the attention of the research community worldwide. There have been multiple evidences which support the role of gut homeostasis in aging. The *Drosophila* gastrointestinal tract is very similar to the mammalian gut, and therefore it can directly be used as a model to understand the association between gut microbiota, immune system, and aging in humans. In current review we have discussed the importance of gut microbiota in aging. Also we have highlighted the importance of host immune system and gut aging. Since the increased microbial load in the gut activates the host immune system, the dysregulated microbiota can have direct implications in gut aging. The proliferation and renewal of intestinal stem cells can also affect gut aging. Another important aspect that we have discussed is the communication between the gut and the other organ systems which affect the overall aging process. Altogether we propose that the *Drosophila* gut can be a good model to improve our understanding of human gut aging.

Keywords

Intestinal homeostasis · Longevity · *Drosophila* · Gut microbiota · Stem cells

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10.1 Introduction

Aging is a pressing challenge with enormous biomedical significance. However, original work, primarily carried out in invertebrate model organisms, has provided insight on the molecular and cellular mechanisms of aging in the last two decades (Gems and Partridge 2013). As a model animal, *Drosophila* has many features which make it most suitable for developmental, neurobiological, and aging studies. A strong hallmark of the *Drosophila* experimental system is that we could pose a variety of questions to understand the basic cell biology and genetics of aging (Piper and Partridge 2017). Moreover, most of the human genes involved in genetic disease are found to have a *Drosophila* counterpart, which has led to important contributions toward human health (Fontana and Partridge 2015). Therefore, *Drosophila* can be used as an efficient model animal to investigate how intestinal tissue homeostasis affects longevity. Recently, the intestinal tissue homeostasis has been found to be associated with its longevity (Gervais and Bardin 2017; Vaiserman et al. 2017).

In *Drosophila*, the gastrointestinal (GI) tract can be subdivided into the crop, foregut, midgut, and hindgut (Fig. 10.1a). The crop is a food storage organ which is equivalent to stomach in the mammals; the foregut to hindgut just serves as the small intestine and colon in mammals (Buchon et al. 2013). To keep the intestinal barrier integrity is very crucial for maintaining the intestinal functions in both humans and flies. When the intestinal functioning is dysregulated, it leads to diseases and aging. The intestinal epithelium is uninterruptedly renewed by intestinal stem cells (ISCs) (Jiang and Edgar 2012).

The intestinal epithelium of *Drosophila* is a monolayer composed of three kinds of cells, most of which are formed by polyploid enterocytes (EC) cells, followed by hormone-secreting enteroendocrine (EE) cells and proliferation monitoring ISCs as shown in Fig. 10.1a (Ohlstein and Spradling 2006). Similarly, ISCs self-renew and differentiate into intermediate cell types, relay amplification cells, multiply and further differentiate into ECs or secretory cells (EEs and Goblet cells), and dedicate the Paneth cell progenitors that develop into Paneth cells (Fig. 10.1b) in mammals. As the signaling pathways regulating the intestinal development and stem cells function maintenance are conserved from *Drosophila* to human (Wang et al. 2014), *Drosophila* intestine can be used as an ideal model to reveal the metabolic pathways, tissue regeneration, ISCs maintenance, host-microbiome associations, and innate immunity for promoting longevity during aging process.

Here, from the insight of *Drosophila* intestines, we discuss the factors that impact the intestinal homeostasis and further regulate the longevity during aging.

10.2 Gut Microbiota and the Host Gut Aging

In the last decade, the relationship between microbiota and host has been substantially uncovered. Microbial community is host specific, and it is established and influenced soon after birth by maternal environment in the early life. Diet, exposure to antibiotics, pathogens, and parasites can also influence compositional features of the microbiota (Sekirov et al. 2010). Surprisingly enough the recent reports have

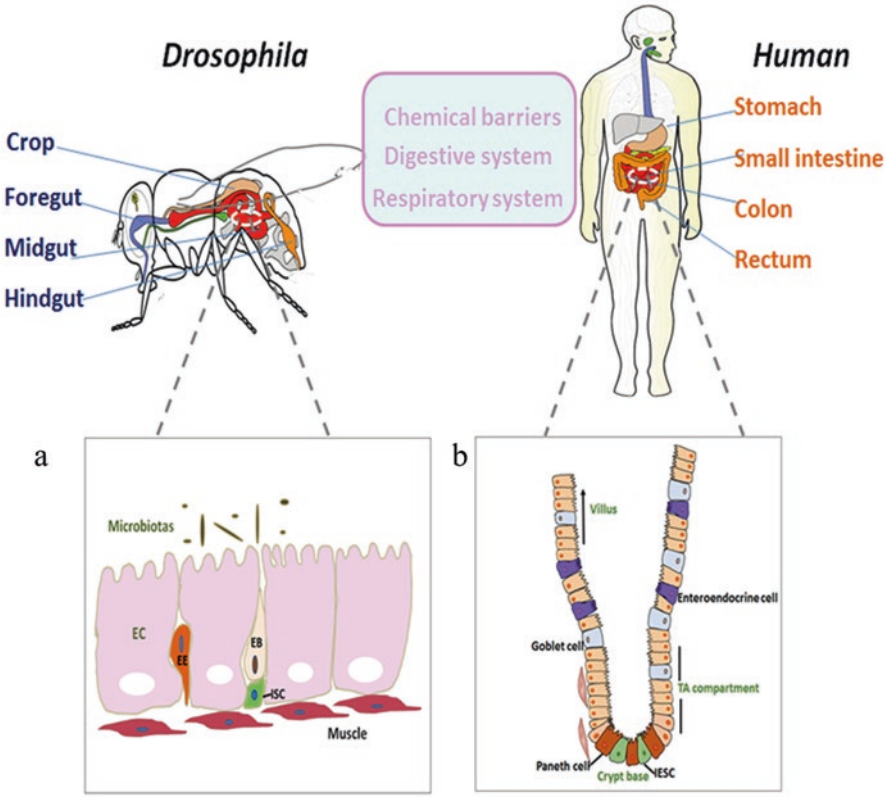

Fig. 10.1 Parallels between the *Drosophila* and human gastrointestinal (GI) tract and gut epithelia. Overview of analogous organ systems in human and *Drosophila* which share evolutionarily conserved defense reactions to prevent and fight such infections. The intestinal epithelium of both humans and flies consists of differentiated epithelial cells, ECs, and EE cells. The regeneration of the gut epithelium from intestinal stem cells that divide asymmetrically to form transient amplifying (TA) cells in humans and analogous enteroblasts (EB) in flies, which then further differentiate into ECs and EEs, showing surprisingly high degree of evolutionary conservation, with homologous signaling pathways being involved

shown that the gut microbiota can have significant involvement in the host's health and disease in numerous ways (Gervais and Bardin 2017; Lee and Hase 2014; Tang et al. 2017; Villanueva-Millan et al. 2015).

In fly, the microbiota has been proven to be involved in the aging process (Clark et al. 2015). A young and healthy *Drosophila* intestine contains a relatively simple microbiota comprising of about 5–20 microbial species and thus is relatively simpler to characterize. The increased bacterial loads have been observed in the guts of aging fly (Fan et al. 2015; Guo et al. 2014), and preventing the accumulation of commensals in the aging gut is sufficient to limit dysplasia. In the young gut, the microbes are mostly restricted to the anterior midgut, but in the old gut, the microbes are transferred to the posterior midgut (Li et al. 2016). Major constituents of these commensals are beneficial microbes, such as *Acetobacter pomorum*, one of the

dominant commensal, recognized by its pyrroloquinoline quinone-dependent alcohol dehydrogenase activity, which modulates IIS in *Drosophila* to regulate host homeostatic programs controlling developmental rate, body size, energy metabolism, and intestinal stem cell activity (Shin et al. 2011).

Lactobacillus plantarum, another commensal, was shown to modulate the target of rapamycin (TOR) pathway, a major sensor of the nutritional status of the cell, and to increase the release of insulinlike peptides (Storelli et al. 2011). Flies overexpressing the inhibitor of TOR complex 1 are resistant to the effects of *L. plantarum* on growth. The reduced intestinal stem cell proliferation observed in axenic flies was also found to be mediated by decreased insulin signaling (Shin et al. 2011). The TOR and insulin pathways play an important role to regulate aging.

Some drugs like rapamycin and metformin having antiaging role have been reported to impact the gut microbiotas. Rapamycin has been thought to target the protein TOR and inhibited its function in *Drosophila*. When the microbial load was checked in the fly guts, it was found that the treatment with rapamycin can slow down the microbial load expansion in the elder fly (Fan et al. 2015; Gaur et al. 2016). Metformin has been reported to alter the gut microbiota composition, for example, an increase of *Escherichia* and a decrease of *Intestinibacter* in the metformin-treated group in the type 2 diabetes (T2D) individuals (Wu et al. 2017) was reported. Also in *Drosophila*, some studies have found that the microbiota interacts with the animal at multiple points in signaling and regulatory networks which is influenced by the nutrition (Dobson et al. 2015).

Primary reports suggested that culturing *Drosophila* anemically shortened the life span, whereas the presence of bacteria within the first week of adult life slows down the aging (Brummel et al. 2004). Moreover, there is now clear evidence that preventing microbial exposure delays the onset of intestinal dysplasia (Broderick et al. 2014; Chen et al. 2014). The single fly FOXO transcription factor-regulated aging induces activation of intestinal genes. In intestinal ECs specific inhibition of FOXO prevents the effects of both age and microbiota on ISC hyper-proliferation (Guo et al. 2014). When the activity of JAK/STAT was reduced, it resulted in improved microbiota homeostasis and extended the life span in *Drosophila* (Li et al. 2016). The state of host-microbe homeostasis is predictive of intestinal barrier function, which in turn is prognostic of fly.

The effect of these bacteria on fly gut is debatable and still under investigation. Additionally, the bacterial genetic makeup is impacted by the signaling of the host or the genotype. Therefore, identifying specific bacterial strains is important to study the fly gut microbiota community and will be useful for studying the health of the fly gut in general.

The members of the *Lactobacillaceae* family have shown conserved probiotic effects from flies to humans, although most *Drosophila* gut commensal species are distinct from those of humans. Therefore, the observations from the *Lactobacillus-Drosophila* interaction model are directly related to the understanding of potential mechanism events of probiotics in more complex vertebrate models, as well as humans. Preventing intestinal dysbiosis without eliminating all the gut bacteria can help maintain the microbial homeostasis and tissue homeostasis and thus promote longevity.

10.3 The Host Immune System Homeostasis, Pathology, and Gut Aging

The *Drosophila* intestine responds to the bacterial infection by host defense and stem cell proliferation (Buchon et al. 2009). As the microbiota load demonstrates expansion in aging guts, the *Drosophila* mobilizes immune system and gut self-regeneration to cope with microbiota expansion.

Most importantly, the gut microbiota plays a crucial role in educating and modulating the host immune system. The intestinal immune systems include physical and chemical barriers, such as pH and digestive enzymes, which provide the first line of defense against invading microorganisms by inducing a variety of inflammatory and antimicrobial responses. The physical barrier of the intestinal tract includes the peritrophic matrix, the shell membrane, and a thin layer of mucus and epithelial barrier integrity.

The immune deficiency (Imd) and Toll pathway are the key components of the response to infection in fly gut. The Toll pathway is the major component of the host response to fungi and Gram-positive bacteria. The Imd pathway recognizes diaminopimelic acid (DAP)-type peptidoglycan found in Gram-negative bacteria and Gram-positive bacilli, as well as peptidoglycan monomers of Gram-negative bacteria. The activity of these pathways can express the production of antimicrobial peptides (AMPs) to remove bacteria (Lemaitre and Hoffmann 2007). RNA-seq analysis has shown that Imd- and Toll pathway-related genes are upregulated in the aging flies (Guo et al. 2014). The negative regulators reduce immune activity by scavenging peptidoglycan (PGRP-LB and PGRP-SC) (Bischoff et al. 2006) or in the case of Pirk by disrupting signaling between the PGRP-LC receptor and Imd (Han et al. 2013; Myllymaki et al. 2014). The intestinal gene *caudal* has been reported to regulate the commensal-gut mutualism by innate immune homeostasis (Ryu et al. 2008). The *Drosophil*a FOXO (dFOXO) has been thought to modulate the metabolism and innate immunity for the AMPs expression (Fink et al. 2016).

The complementary immune mechanisms are active in the gut which are mediated by the production of reactive oxygen species (ROS) by NADPH oxidase enzyme Duox (Guo et al. 2014; Ha et al. 2005). In *Drosophila*, genetic evidence shows Duox-dependent ROS participate in several aspects of intestinal microbial response, such as microbial scavenging, redox-dependent regulation of signaling pathways, intestinal stem cell activation, and cross-linking of the peritrophic matrix (Kim and Lee 2014). It was also observed that the flies lacking Duox activity are more susceptible to infection with enteric pathogens and have increased mortality in the presence of dietary bacteria and yeast (Chakrabarti et al. 2014).

Therefore it can be stated that the elevated immune activity caused the intestinal hyperplasia in the aging guts. As the IMD shares numerous features with mammalian tumor necrosis factor, fly gut could be an interesting model to study the effect of intestinal hyperplasia during aging in mammals.

10.4 The Gut Stem Cells Self-Renewal Impact the Aging

Low levels of turnover are commonly observed under normal, homeostatic conditions in guts. ISC proliferation rates are significantly increased in response to the chemically induced damage or pathogenic bacterial infection. Although adaptive ISC divisions can maintain tissue homeostasis through the replenishment of lost or damaged cells, uncontrolled ISC division and altered differentiation programs can lead to loss of tissue function (Fig. 10.2). In the *Drosophila* midgut, aging results in the consistent display of several ISC-related phenotypes, including an increase in ISC proliferation and a block in fatal differentiation of ISC offsprings, as reflected by the accumulation of polyploid cells that express the ISC/EB marker Escargot (Biteau et al. 2008).

Epithelial dysplasia is caused by both over-proliferation and mis-differentiation of ISCs. JNK, EGF, insulin/IGF (IIS), JAK/STAT, and p38MAPK signaling pathways are essential for ISC proliferation (Ayyaz and Jasper 2013; Biteau et al. 2011; Buchon et al. 2009). Long-term stem cell maintenance is guaranteed by mechanisms that prevent activation of target of rapamycin (TOR) signaling (Amcheslavsky et al. 2014). Stressors that trigger ISC proliferation include oxidative stress, bacterial infection, DNA damage, and aging.

The intestinal stem cells (ISCs) determine the potential of intestinal self-healing integrity. In the intestine of *Drosophila*, aging is correlated with an increase in ISC proliferation (Jiang et al. 2009). Over-proliferation and mis-differentiation of ISCs caused epithelial dysplasia (Jiang et al. 2009; Regan et al. 2016). A current review of the transcription of each type of intestinal cell represents a significant change in gene expression during infection: 1833 genes were differentially expressed in ISCs, 233 in ECs, 433 in EEs, and 2646 in EBs (Dutta et al. 2015).

So, this leads to alterations in localization of cell-cell junctional complexes, loss of the typical apical-basal organization of the epithelial monolayer, and a decline in intestinal barrier function. The dysfunction of the gut speeds up the aging process and shortens the life span in *Drosophila*.

10.5 Gut Aging and Organ-to-Organ Communication

There is now substantial evidences suggesting that several non-gastrointestinal organs and tissues interact with the gut to ensure the homeostasis state of the organism (Amcheslavsky and Ip 2012; Mayer 2011). The first good neighbors for the gut are the hemocytes, which are associated with the phagocytosis and encapsulation of invading pathogens. Hemocyte-expressed decapentaplegic (DPP) triggers Saxophone (SAX) and Smad on X (SMOX)-activated epithelial cells in the early stages of infection, promoting the proliferation of ISC and rebuilding ISCs tranquil need to activate Thickvein (TKV) and MAD. The adults with hemocyte loss are sensitive to infections. Precisely, hemocyte-expressed Unpaireds (Upds) are pertinent to ISC mitogenesis after infection, emphasizing a role for hemocytes in intestinal regeneration.

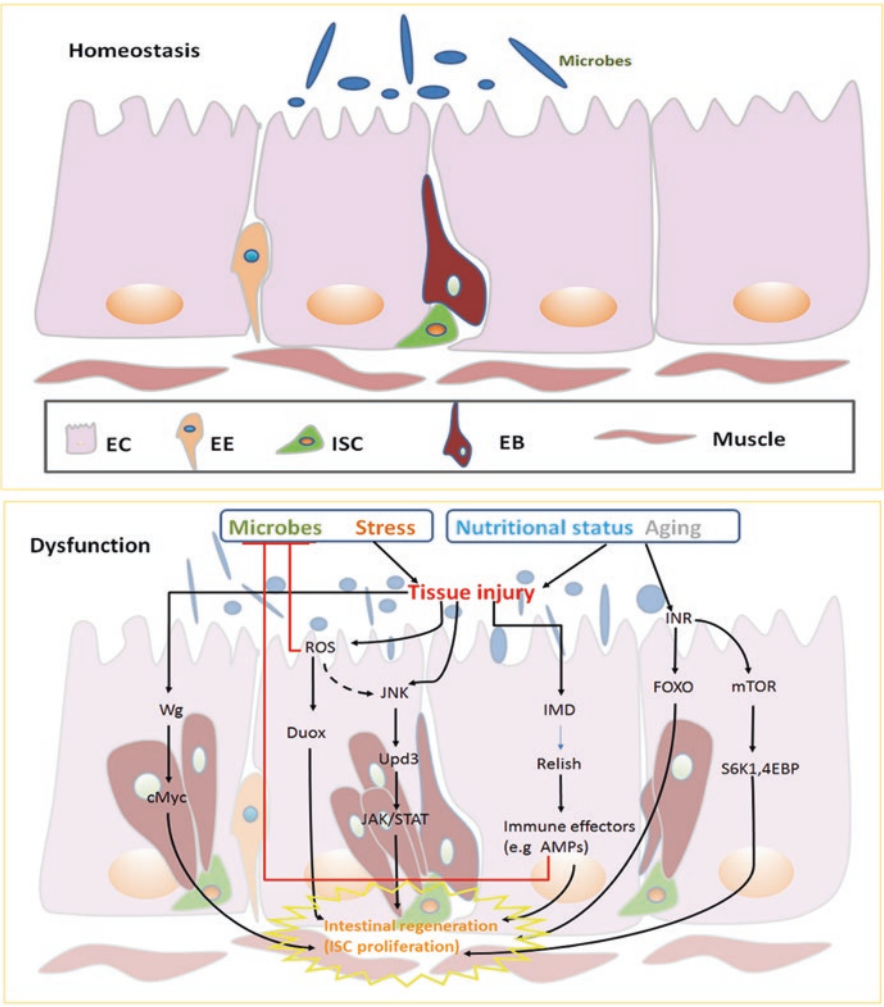

Fig. 10.2 The homeostasis and dysfunction of the *Drosophila* midgut and the signaling to regulate the intestinal regeneration. In homeostasis guts, the epithelium consists of a monolayer of ECs with interspersed EEs and basally located ISCs. The microbes and other stress can lead to injury in the guts and the gut epithelium products' immune effectors, including reactive oxygen species (ROS) and antimicrobial peptides (AMPs). In *Drosophila*, activation of the JAK-STAT pathway by the cytokine Unpaired 3 (Upd3) triggers induces the proliferation of stem cell. The activation of JAK-STAT also directly stimulates the differentiation of ISC. The wingless (WNT/WG) pathway is a major regulator of the ISC proliferation in the mammal and also through cMyc to promote tissue regeneration in the infected *Drosophila* midgut. The nutrition status and aging regulated the intestinal regeneration mostly by IIS/mTOR pathways. The dashed arrows indicate presumed activities but as yet are undefined. Epithelial cells and immune cells secrete cytokines that stimulate tissue regeneration. The over-proliferation of ISCs, resulting in the accumulation of misdifferentiated EB-like cells, disturbs the structure and function of the intestinal epithelium. This phenotype is associated with the expansion of the symbiotic bacterial population in the cavity

Study of signaling pathways involved in the fat body-gut communication model is a productive research tendency. Many studies focused on gut-mediated metabolic homeostasis in the fat body. The importance of gut-expressed signals in energy metabolism is particularly highlighted by some interesting studies. One such study reported that overexpression of PGC-1 in the intestine significantly increases both the free glucose levels and amount of stored glycogen, along with a decrease in triglyceride (TAG) levels, suggesting that PGC-1 plays the role in maintaining energy homeostasis. Also the intestine-specific overexpression of PGC-1 extended life span (Rera et al. 2011). This may be a key strategy for fat body signaling to the gut, providing a potential link to the fat body expression factors and intestinal behavior.

This could be a critical strategy by which the fat body signals to the intestine, providing a potential link between fat body-expressed factors and intestinal actions. Zheng and co-workers have demonstrated the involvement of lamin B from fat body cells in gut hyperplasia during aging (Chen et al. 2014). The fat body-specific loss of lamin B aggravates immunosenescence, which contributes to the dysregulation of the Imd signaling pathway, causing turnover loss in the intestinal epithelium (Chen et al. 2014, 2015).

Moreover, the fat body secretes antimicrobial peptides (AMPs) to directly remove ingested pathogens; the intestinal immune response also affected the general AMPs. The AMP expression levels in the fat body are related with the gut-expressed PGRP-LE, which can be suppressed by numerous gut-expressed amidase peptidoglycan recognition proteins (PGRPs), such as PGRP-SCs and PGRP-LB (Costechareyre et al. 2016; Paredes et al. 2011). It is recommended that the gut-fat body communication regulates and controls universal AMP production, which is equal to AMPs from the intestine and contributes to the defense against ingested pathogenic microorganisms. Taken together, these important results show that the signal from the fat body controls intestinal activity in several aspects, including intestinal inflammation and metabolic homeostasis in the body.

Over the past few years, the brain-gut communication model has been an intensive area of investigation. The brain directly affects intestinal immunity, physiology, and metabolism (Nässel et al. 2013). A study has shown that brain-expressed Dilp2 is essential for ISC self-renewal after damage. Loss of brain-expressed Dilp2 caused an increased number of phospho-histone3+ (PH3+) cells under inflammation (Amcheslavsky et al. 2009). CREB and CRTC inhibition induces the loss of short neuropeptide F (SNPF) in ECs, resulting in imbalance of epithelial integrity, ampere level, and energy balance defects (Shen et al. 2016). *Ulgherait* et al. have detected the role of AMPK in autophagy in the brain and gut. They showed that overexpression of AMPK in the brain activated autophagy and slowed the intestinal aging. They also suggested that the activity of autophagy-specific gene 1 (*Atg1*) which is expressed in the brain is associated with the maintenance of intestinal homeostasis (Ulgherait et al. 2014).

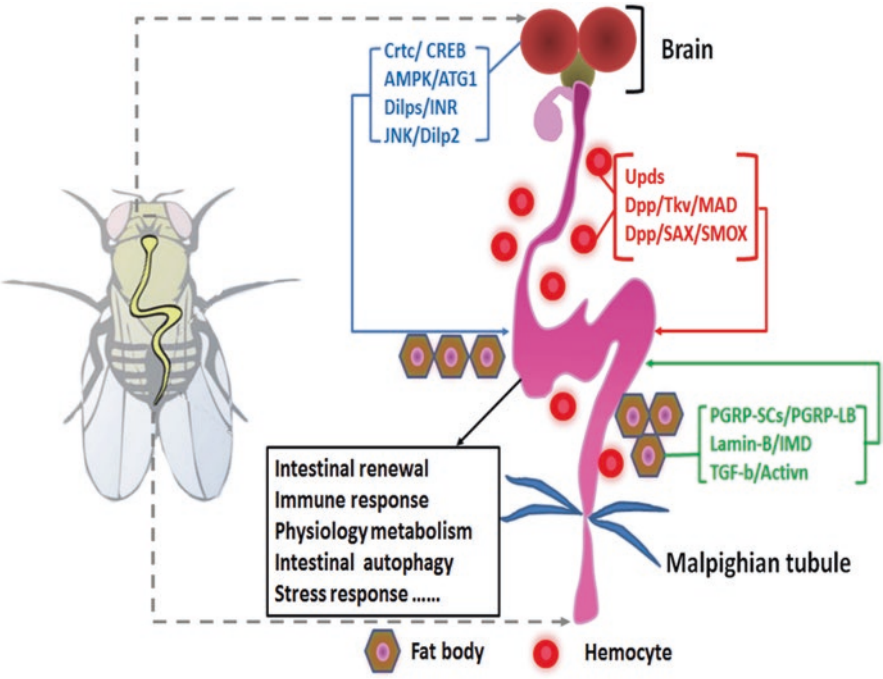

Fig. 10.3 Diagram of gut-neighbor organ communication in adults *Drosophila*

In summary, the reason behind this correlation between the gut and neighboring organs is very complex, and the consequence of this cross-talk in response to aging is even more intricate. The neighbor organs might be involved in gut aging by expressing the genes involved in metabolism, stress response, and life span (Fig. 10.3).

10.6 Remark

Here we reviewed the gut homeostasis and the *Drosophila* longevity. The gut works as a barrier and the first line of defense against swallowed toxins and pathogens and also provides an accommodating environment for microbes that help digestion. More interestingly it is one of the sites in *Drosophila* that harbors a pool of active stem cells. Therefore, the homeostasis of the gut is important to keep the health span and life span in *Drosophila*. The microbes, the body immune system, and the neighbor organs collaborate to regulate the homeostasis of intestinal microenvironment during the aging. When the synergistic abilities of these factors get weaken in the course of aging, the intestinal tract is not able to maintain the normal rate of renewal, resulting in dysfunction of intestinal. At present the degree to which gut dysfunction is generally limiting the life span is not clear.

Whatever the situation, the gut is an important place to understand the details of how aging alters stem cell function, as well as the more complex association between aging microbiota and the immune system. We have also discussed the advantages of having *Drosophila* as a model system. Studies on the *Drosophila* gut have been critical in developing our current understanding of the molecular basis of aging, and this will also improve our understanding of human aging.

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Aging Kidney and Aging-Related Disease

11

Zhongchi Li and Zhao Wang

Abstract

With the development of society and improvement of health care, the life span is much longer than before, which brings serious aging problems. Among all the aging problems, renal aging grows to be nonnegligible issue. The aging process of kidney is always accompanied with structural and functional changes. Molecular changes, including Klotho and Sirtuins, are the basic causes of phenotypical changes. Cell senescence and cell autophagy play fundamental roles in the process of renal aging. To effectively intervene in the process of renal aging, different methods have been tried separately, which could produce different effects. Effective intervention of renal aging could be meaningful for healthy state of the whole body.

Keywords

Renal aging · Autophagy · Cell senescence · Epigenetic regulation

11.1 Introduction

While life expectancy in most countries has constantly increased in the last century, resulting from the improvements in sanitation and health care, the proportion of older people in the general population is continuously increasing. It is predicted that

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there will be 72 million 65-year-old or older Americans, which account for around 20% of the population by 2030 (O'Sullivan et al. 2017). In China, the number of 60-year-old or older people is estimated to be 374 million by 2040, comprising 24.8% of the Chinese population (Zhou et al. 2008a). By 2050, the population that is older than 60 years old will likely outnumber those younger than 14 years and constitute two billion people in the world (Zhou et al. 2008b). The rapid increase of geriatric population would bring a series of medial, social, and economic problems. A wise response to the increase aging population would require finding out the origin of this phenomenon, catching on the opportunities, as well as solving the problems effectively.

An essential geriatric medical issue is that of aging-related renal disease (Buemi et al. 2005). Although the aging process does not cause renal disease directly, the kidney goes through obvious physiologic change during the aging process, which predispose kidney to kinds of pathology. Although those diseases are not specific to the elderly, some certain disorders are more prevalent in the elder population, for example, renal disease secondary to type 2 diabetes mellitus (Silva 2005a). The impact of aging on the whole body showed impaired maintenance of physiological homeostasis and increasing burden of "wear and tear." Therefore, aging kidney is not an independent disease but a complicated state owing to accumulative stress. Renal aging is normally mixed with a series of diseases, which makes it hard to distinguish normal features of the aging process from age-related dysfunction. This phenomenon is pretty common in a range of morbidities and is particularly relevant in the context of chronic kidney disease (CDK). CDK is an important public health problem that is characterized by poor health outcomes and very high health-care costs. CDK is also a major risk multiplier in patients with diabetes, hypertension, heart disease, and stroke (Couser et al. 2011). The incidence of CKD is about 10–12% in the developed world, and it is still increasing (Levey et al. 2015).

Aging in most species associates with impaired adaptive and homeostatic mechanisms, which induce an organism susceptible to inner or outer stresses (Anderson et al. 2009).

There is no gold standard for determining what constitutes normal aging. Although a loss of physical capability and function is induced by increasing chronological age, solid differences exist among people of the same chronological aging, which owes to genetic and epigenetic factors. However, over the past 50 years, a series of candidate biomarkers of aging have been found, even though they are not sufficient to prove aging (Baker and Sprott 1988). There are nine hallmarks of aging, including genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication (Lopez-Otin et al. 2013). As for renal senescence, the typical histologic features are decreasing cortical mass, increases in glomerulosclerosis, interstitial fibrosis, tubular atrophy, and arteriosclerosis (Zhou et al. 2008b). In terms of renal function, elderly individuals usually exhibit increased renal vascular resistance, reduced renal plasma flow, and increased filtration fraction (Fliser et al. 1993). There are several reviews talking about the role of cell senescence in renal aging. Some features of cell senescence, such as the appearance of senescence-associated β-galactosidase (SA-β-gal) and P16INK4a, appear before showing

morphologic changes, which suggests cell senescence may be part of the reason for renal diseases. To discover efficient method attenuating renal senescence, it is necessary to understand the factors driving age-associated changes in kidney.

11.2 Features of Renal Senescence

11.2.1 Structural Changes of the Aging Kidney

The structural changes of the aging kidney mainly include a decline in total nephron size and number, tubulointerstitial changes, glomerular basement membrane thickening, and increased glomerulosclerosis and so on (Nyengaard and Bendtsen 1992), as shown in Fig. 11.1. The average kidney weight progressively declines after 50 years old, and the renal cortex is more vulnerable to the aging-related stress than the medulla (Zhou et al. 2008b). Decreasing glomeruli are always accompanied by decreasing kidney weight and thinning of the cortical ribbon during aging (Nyengaard and Bendtsen 1992; Hughson et al. 2006; Newbold et al. 1992). Many morphologic changes have been noted before, including progressive decline in the number of intact or normal glomeruli with age, increase in the number of globally sclerotic glomeruli, abnormal glomeruli with shunts between the afferent and

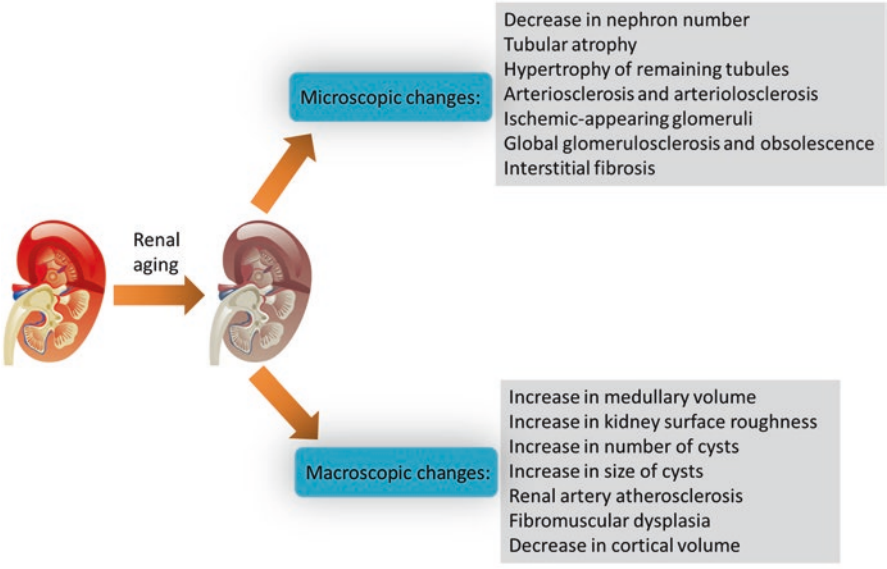

Fig. 11.1 Structural changes of the aging kidney. The structural changes of the kidney can be classified into two ways. Firstly, at the microscopic level, nephron number decreases significantly and some of tubules atrophy, while some of them hypertrophy. Arteriosclerosis and interstitial fibrosis are common in the aging kidney. Sclerosis and obsolescence appear in the glomerulus. Secondly, at the macroscopic level, medullary volume, kidney surface roughness, and number and size of cysts are all increased in the aging kidney, while cortical volume decreased. Besides, renal artery atherosclerosis and fibromuscular dysplasia are both typical in the aging kidney

efferent arterioles bypassing the glomeruli, progressive decrease in the size of intact glomeruli, focal or diffuse thickening of the glomerular basement membranes, and increased mesangial volume/matrix-sclerosis (Silva 2005b). The pathogenesis of aging-associated global glomerulosclerosis is multifactorial. Dysregulation of the afferent and efferent arterioles of the glomerulus may lead to increased glomerular plasma flow, increased glomerular intercapillary pressures, and subsequent "hyperperfusion" glomerular injury with mesangial matrix accumulation (Brenner 1983).

It is reported that the vascular changes seen in aging kidneys first cause cortical glomerulosclerosis and consequent juxtamedullary glomerular hypertrophy, followed by juxtamedullary glomerulosclerosis (Newbold et al. 1992). Cadaver studies estimate that the upper limit of normal glomerulosclerosis in aging exceeds 10% (Chan et al. 1990). Normally, severe glomerular inflammation and crescent formation always incur a series of abnormities, including sclerosed glomeruli with breaks in the glomerular basement membrane, which should be paid attention to the possibility of underlying IgA nephropathy, antineutrophil cytoplasmic antibody-associated vasculitis, or lupus nephritis (Glassock and Rule 2012).

Tubular changes in the aging kidney are obvious, including a decrease in the tubular volume and number; a decrease in the tubular length and increased numbers of tubular diverticula, especially of the distal convoluted tubules; tubular atrophy, often with implication of tubular epithelium with thickening of the tubular basement membranes; and increase in the interstitial volume with interstitial fibrosis and sometimes even inflammatory cells (Silva 2005b). Tubular atrophy and interstitial fibrosis may be aging-related or may occur due to chronic inflammation or vascular disease (Nadasdy et al. 1994). Normally, there are three types of tubular atrophy, including the classic form with wrinkling and thickening of the tubular basement membranes and simplification of the tubular epithelium, thyroidization form with hyaline cast-filled dilated tubules, and the endocrine form with simplified tubular epithelium, thin basement membranes, and numerous mitochondria (Glassock and Rule 2012). These three types of tubular atrophy can be taken as a result of incomplete healing due to any type of renal injury. Among people over 40 years, it is common to see simple renal cysts, which might derive from the diverticula in distal and collecting tubules, probably promoting bacterial growth and contributing to the frequent renal infections in the old individuals (Tada et al. 1983).

As for renal vasculature, there are also a number of changes noted in the aging kidney. Those changes include tortuous interlobar arteries with thickening of the medial muscle cell basement membranes, "fibroelastic hyperplasia" of the arcuate and subarcuate arteries, myointimal fibrosis of the interlobular arteries, and hyaline change insudation of the afferent and the efferent arterioles (Gibson et al. 1996). "Arterial sclerosis" denotes thickening of the arterial wall and narrowing of the vascular lumen produced by thickening of the medial muscle cell basement membrane, fibrosis of the media, or intimal thickening, and may be seen with hypertension, diabetes mellitus, and aging, with the frequency of the arterial sclerosis increasing with advancing aging (Silva 2005b).

11.2.2 Function Changes of the Aging Kidney

It is reported that the glomerular filtration rate (GFR) begins to decline at a rate of 8–10 ml/min/decade after 30 years old (Morrissey and Yango 2006). However, there are still one-third of elderly individuals showing no change in GFR. This variability suggests that there should be other factors besides aging responsible for apparent reduction in renal function. Increased mean arterial blood pressure correlated directly with more aggressive decline in the aging process (Lindeman et al. 1985). Besides, low high-density lipoprotein cholesterol is also associated with accelerated age-related loss of renal function (Sesso et al. 2008). However, it is not clinically significant of the decline in renal function until some diseases further impaired the renal structure or function in the aging individuals. Besides traditional clearance of infused exogenous filtration markers, including insulin and iothalamate, creatinine clearance in a timed urinary sample is used as an estimate of GFR (Maher et al. 1971). However, creatinine clearance is affected by the nutritional status, protein intake, and muscle mass, which makes it not accurate to estimate the GFR in the elderly (Kimmel et al. 1996). The urinary creatinine output and creatinine production decline gradually with the aging-related decrease in muscle mass and body weight proportionately (Musch et al. 2006). Therefore, the serum creatinine does not increase with time in the old individuals, even in those with mild azotemia (Silva 2005a). Coresh has suggested: "Methods to estimate risks of CrCl for older subjects as a function of age and race in large epidemiologic studies are not properly standardized or of sufficient accuracy raising the question of validity in estimating renal function and therefore better methods to estimate CrCl are strongly encouraged."

Under normal conditions, renal vasodilation leads to a significant increase in the renal blood flow and GFR, representing renal hemodynamic and functional reserves. The increase in the renal plasma flow (RPF) and GFR in response to maximum renal vasodilation induced by concurrent infusion of amino acids and dopamine is markedly reduced in healthy elderly individuals (Fuiano et al. 2001). A study involving 207 healthy kidney donors showed an explicit progressive reduction in mean blood flow per unit mass with aging, which suggests that the decrease in RBF does not simply reflect decrease in the renal mass with aging (Hollenberg et al. 1974). The reduction in RPF induced by intravenous administration of the nitric oxide (NO) synthase (NOS) inhibitor increased markedly with aging in men but not in women. Increasing plasma levels of endogenous NOS inhibitor and associated NO deficiency are shown to contribute to the aging-related decline in RPF (Kielstein et al. 2003a). In aging, there is a tendency for the response to vasodilators, for example, endothelial-derived hyperpolarizing factor (EDHF) to be attenuated, while the responsiveness to vasoconstrictors like angiotensin II (A-II) is enhanced, which might lead to enhanced vaso-constructive responses in aging, potentially causing renal damage and ultimately a fall in GFR (Long et al. 2005).

Due to fatigue and fracture of the medial elastin, most of the elastic arteries showed changes with aging but little changes in distal muscular arteries (Virmani

et al. 1991). Increased arterial stiffening results in an increase in pulse wave velocity (PWV). Aortic PWV is the speed with which pulse wave travels along the wall of the artery, and there is a 2.5-fold increase of the value comparing 80-year-old and 20-year-old individuals (Zhou et al. 2008b).

Changes of renal function during aging represent in many ways. Firstly, abnormal sodium balance and slower homeostatic responsiveness that result from the inability to conserve and excrete sodium chloride are common in the elderly as compared with young individuals (Kielstein et al. 2003b). Inability to conserve sodium may predispose the elderly to hemodynamic instability in the setting of sodium loss (Shannon et al. 1986). Due to a decline in the countercurrent maintenance of the medullary hypertonicity, there is a significant decline of the ability to maximally dilute or concentrate urine, which might cause volume depletion and dehydration as well as abnormal osmolar states. Compared with healthy young subjects, the mean lithium clearance that is an indicator of proximal tubular function was much lower in the elderly. Besides, the fractional proximal sodium reabsorption was much higher in the elderly (Fliser et al. 1997). The ability of the kidney to both maximally concentrate and dilute the urine deteriorates with age, which makes older individuals more susceptible to the development of delusional hyponatremia in the setting of excess water load (Rowe et al. 1976). Reduced renal diluting capacity also predisposes the elder to stress situations such as surgery, fever acute illness, and administration of drugs like diuretics or others that enhance vasopressin action. The susceptibility to drug toxicity is partly due to the altered drug pharmacokinetics that stems from a decline in the functional capacity of the kidney, as well as altered the body composition, which is popular among the elderly. Furthermore, aging affects pharmacodynamics of many drugs by modulating the sensitivity and physiological response to their actions. The homeostasis of acid is also regulated by the kidney through conserving bicarbonate and by net acid secretion as ammonium and titratable acid in the renal tubular lumen. Normally, the kidneys of the elderly could function similar to those in young subjects. However, once challenged with acute acid load, the senescent kidneys do not increase acid excretion and lower urinary pH efficiently as those of younger kidneys (Luckey and Parsa 2003). Age-related mild metabolic acidosis develops apparently partly due to normal age-related decline of renal function, although the blood pH and serum bicarbonate of the geriatric population without renal disease do not differ significantly from the young under basal conditions (Frassetto et al. 1996).

Decreasing renal function causes prevalence of anemia, which is related to the reduced erythropoietin (EPO) production by the kidney (Eisenstaedt et al. 2006). The prevalence of anemia increases with age. It has been reported that there is a trend toward an increase in prevalence of anemia with decreasing renal function (Ble et al. 2005). Serum EPO levels rise with age in healthy subjects, perhaps a compensation for aging-related subclinical blood loss, increased red blood cell turnover, or increased erythropoietin resistance of red cell precursors (Ershler et al. 2005). The kidney could remove around 50% of insulin from the systemic circulation, which is accomplished by glomerular filtration and proximal tubular uptake and degradation. Whereby, decreased kidney function leads to reduced insulin

clearance ability. Some research suggested that the impaired insulin secretion of aging rats might not result from old age but might be associated with chronic renal insufficiency and excess of parathyroid hormone (Massry et al. 1991). Insulin secretion was actually impaired in the elderly individuals, and total body insulin clearance is lower in the elderly than in the young subjects. In a study, elderly women with osteoporosis and low creatinine clearance had lower calcium absorption, lower serum 1,25-dihydroxyvitimin D, and normal serum 25-hydroxyvitamin D, suggesting decreased conversion between them by the kidney (Gallagher et al. 2007). Besides reduced activation of vitamin D, elderly individuals also demonstrate increased renal calcium loss from reduced calcium reabsorption in the distal convoluted and connecting tubules (Arnaud and Sanchez 1990).

11.2.3 Autophagy and Cell Senescence in the Aging Kidney

Autophagy has been proved to be essential for many fundamental biological activities, such as cellular stress response and maintenance of cellular homeostasis (Wang and Klionsky 2011). Dysregulation of autophagy is involved in the pathogenesis of a variety of metabolic and age-related diseases (Shibata et al. 2006). The role of autophagy in the diseased kidney has been studied mainly in proximal tubular cells and podocytes. The most fragile cells in glomerulus have been identified to be podocytes, and a decreased number of podocytes is directly correlated with disease progression in several renal diseases (Ziyadeh and Wolf 2008). Glomerular endothelial cells are also altered in several renal diseases. During aging, it is common to see accumulation of damaged organelles and mitochondria, which will cause organ dysfunction. The kidney is particularly susceptible to age-related renal damage, such as tubular atrophy, interstitial fibrosis, and glomerulosclerosis. The elderly are more sensitive to ischemia and toxic stress and show high rates of end-stage renal disease and chronic kidney disease (Nitta et al. 2013; Bolignano et al. 2014). It is reported that autophagy is the mechanism for kidney clearing those damaged components in podocyte, which could maintain their capacities for regeneration. Furthermore, podocytes have a high level of basal autophagy, which suggests autophagy is essential for this kind of cells.

There are a series of causes for acute kidney injury (AKI), including sepsis, xenobiotic agents, drugs, and ischemia-reperfusion. Autophagy has been proved as a renoprotective cellular response in AKI (Huber et al. 2012). The renal tubulointerstitial compartment is not spared from aging-induced damage. Tubular atrophy, apoptosis, and fibrosis are associated with a decline in the filtration function in the aged kidney. Some research found that mice with a specific autophagy deficiency in renal proximal tubular cells have increased accumulation of damaged mitochondria and ubiquitinated proteins, which suggests that autophagy is protective in proximal tubules cells during aging and autophagy deficiency in renal tubular cells leads to premature aging (Liu et al. 2012; Kimura et al. 2011). According to these results, autophagy activation in the aging kidney would protect from age-related renal dysfunction.

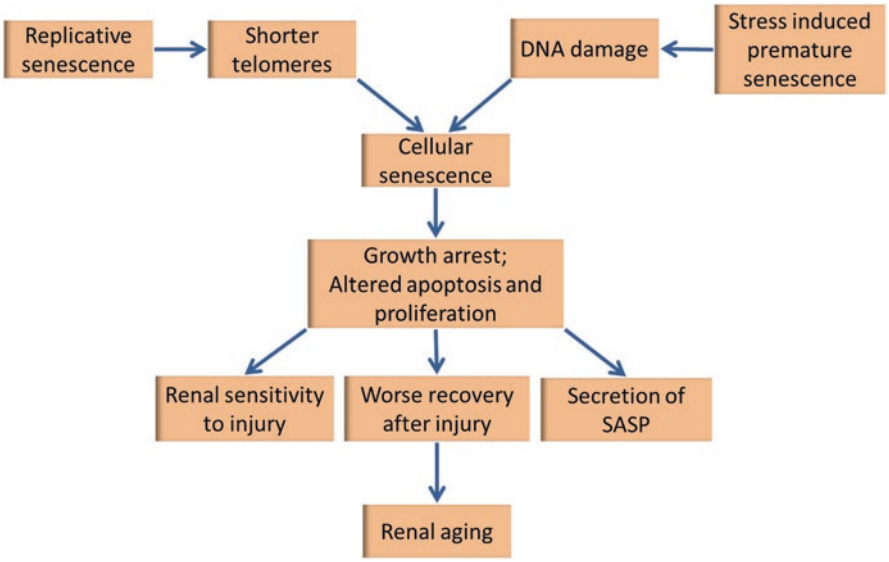

Fig. 11.2 The relationship between renal aging and cell senescence. Both shorter telomeres and DNA damage are main causes of cellular senescence. Compared to normal cells, senescent cells have arrested growth, which manifests disordered proportion of apoptosis and proliferation. The kidneys containing senescent cells are more sensitive to inner or outer injury and recover more slowly after injury, which results in many complications. Senescence-associated secretory phenotype (SASP) is secreted by senescent cells, which affect negatively other surrounding cells, inducing aging of the whole kidney

Cell senescence is not only a marker of renal aging but also a participant, as shown in Fig. 11.2. Development senescence is thought to be induced as part of a physiological program that promotes cell replicative senescence. Besides, some other pathologic stresses, such as mitochondrial injury and oxidative stress, could activate p53 or p16 pathway. The growth of senescent cells has arrested, and the balance between apoptosis and proliferation has also broken down. Senescent cells obtain distinct phenotypes, including chromatin modifications and profound changes in protein secretion, which are referred to as the senescence-associated secretory phenotype (SASP). The SASP is a critical characteristic of senescence programs. SASP factors with developmental functions remain to be identified. Chronic senescent cells acquired with aging or following treatment are highly variable depending on the type of stressor, tissue, and species; however, they consistently induce IL-6 and plasminogen activator inhibitor I in vivo. So far, IL-6, IL-1a, plasminogen activator inhibitor 1, and MCP-1 are taken as SASP factors induced by senescent cells (Yang and Fogo 2010).

11.2.4 Molecular Changes in the Aging Kidney

The Klotho gene was originally identified as a gene mutated in a mouse strain, which exhibited a syndrome resembling premature aging. In contrast, overexpression of the Klotho gene extended life span in the mouse, which suggests Klotho gene may function as an aging-suppressor gene (Kuro-o et al. 1997). Other research has found Klotho gene is related to osteoporosis, stroke, and coronary artery diseases (Arking et al. 2002). The Klotho gene is expressed notably in the distal convoluted tubules in the kidney and the choroid plexus in the brain. The multiple aging phenotypes caused by mutation of Klotho are mediated by its hormonal action via its binding to a cell-surface receptor and repressing the intracellular signals of insulin and insulin-like growth factor 1. The inhibition of insulin/IGF-1 signaling by Klotho is associated with increased resistance to oxidative stress (Kurosu et al. 2005). It has been demonstrated that renal Klotho mRNA is downregulated under sustained circulatory or metabolic kidney disease (Koh et al. 2001). Angiotensin-II might be the reason of decrease of renal Klotho gene expression during aging, which may be mediated through promoting intrarenal iron deposition and inducing oxidative stress (Saito et al. 2003).

Telomeres in somatic cells shorten with each cell division, and this progressive attrition leads to critically short telomeres and cellular senescence (Jiang et al. 2007).

It is commonly believed that telomeres act as a mitotic clock, initiating replicative senescence when telomeres become short enough after a certain number of divisions. The enzyme telomerase is required for the maintenance of the size and stability of the length of telomeres. Senescent cells express p16 and p21, inhibiting cellular proliferation by inhibiting cyclin-dependent kinases (Hara et al. 1996). There is an overall increase in p16 expression in the elderly compared with young individuals, particularly in the renal cortex, although this expression varies among different individuals (Chkhotua et al. 2003).

Sirtuins are a series of NAD^+-dependent deacetylase associated with numerous aspects of health and physiology. Among the seven members, Sirt1, 3, and 6 have been reported to function positively in the renal aging. The renal protective effects of Sirtuin are found in various models of renal disorders with metabolic impairment, such as diabetic nephropathy. Sirt1 could exhibit histone deacetylation activity and renal protective effects through regulation of various factors such as p53, NF-κB p65 subunit, STAT, FoxO1, and FoxO3, which are transcriptional factors related to apoptosis, cellular aging, and inflammation (Wakino et al. 2015). The expression of Sirt6 decreases significantly during aging process in mice. High-fat diet, an aging-promoting factor, could reduce expression of Sirt6 in the kidney (Zhang et al. 2016). Recently, it has been proved overexpression of Sirt6 could protect the kidney from cisplatin-induced acute kidney injury, and absence of Sirt6 would aggravate the injury caused by cisplatin (Li et al. 2018).

Increased oxidative stress and accumulation of free radicals are believed to play an essential role in the process of aging. Lipofuscin is free radical damaged proteins and fat, and it is insoluble and not degradable by either lysosomal enzymes or the proteasomal system. The release of lipofuscin and lysosomal contents cause cell

damage and dysfunction (Jung et al. 2007). The increase in oxidative stress and lipid peroxidation in the aging kidney is related to an increase in the advanced glycosylation end products and their receptors, which might degrade hypoxia inducible factor-1a (HIF-1a) and limit the capacity of the aging cells to form hypoxia inducible factor-1-DNA hypoxia-responsive recognition element complexes. In the kidney, the consequent decrease in the ability of the cells to respond to hypoxia could explain the attenuated anemia-induced secretion of erythropoietin (Frenkel-Denkberg et al. 1999).

To elucidate the intricate relationship among diverse pathways, which are involved in the aging process, researchers performed a whole-genome analysis of gene expression in kidney samples from 74 patients ranging from 27 to 92 years old. In the results, more than 900 aging-related genes are found to change, and there are small changes of transcriptional differences of many genes, rather than huge changes of several essential genes, which suggests aging is a complex process involving a large number of intimately related molecular pathways (Rodwell et al. 2004).

11.3 Intervention Strategies of Renal Senescence

11.3.1 Clearance of Senescent Cells

The goal of any therapeutic intervention improving the features of kidney aging is the preservation of long-term kidney health and function. It emerges a new field in aging research intending to reduce the number of senescent cells. To clear the senescent cells efficiently and avoid potential off-target effects, identifying the differences between senescent cells and non-senescent cells is critically important. Senolysis could be best achieved with strategies similar to those used to kill cancer cells, such as activation of the immune system, inhibition of pro-survival pathways, or activation of pro-apoptotic pathways. In most organs, senescent cells should be cleared by immune system, whereas some chronic senescent cells could resist clearance in different contexts (Hoenicke and Zender 2012). The adaptive immune system is essential in recognizing premalignant hepatocytes, and the innate immune response is particularly important for the physiological removal of senescent cells. The SASP of senescent liver cells activates macrophages and induces their polarization toward the secretory M1 type, which ultimately leads to senescent cells clearance (Lujambio et al. 2013). On the contrast, it will cause increased liver fibrosis when the physiological natural killer cells are impaired, which leads to low efficiency of clearance of activated stellate cells in livers (Sagiv et al. 2016). Therefore, we have the reason to conclude that pharmacological agent increasing the susceptibility of senescent cells to immune cell-mediated clearance could be an efficient way improving aging-associated diseases.

Although it seems to be beneficial to remove renal senescent cells in many kidney diseases and renal aging, the effect has not been confirmed yet. It is found that during the healing period after implantation, senescent cells would promote cutaneous wound healing (Demaria et al. 2014).

Inducing autophagy could be an effective way to clear senescent cells, which might be a promising therapeutic strategy for the treatment of renal diseases. However, the protective effect of autophagy in renal diseases is still lacking for human diseases. Autophagy inducers commonly used in human medicine are mTOR inhibitors, such as sirolimus, everolimus and rapamycin. They are normally used as immunosuppressive agents, but they have strong adverse effects on compensatory renal epithelial cell hypertrophy and recovery from ischemia following renal transplantation (Pallet and Legendre 2013; McTaggart et al. 2003). Therefore, it seems not to be a feasible therapy to use mTOR inhibitors as autophagy inducers for renal diseases. It is promising to discover a specific approach that enables pathway-specific and kidney-selective regulation of autophagy, which could minimize the side effects.

11.3.2 Modifying the Epigenome

More and more evidences have shown up from model organisms to humans, indicating that psychosocial interventions, nutrition, and even exercise can interfere aging process (Simpson et al. 2010; Epel et al. 2004; Blackburn et al. 2015; Fontana and Partridge 2015). Furthermore, those interventions could target pathways that are essential in the epigenetic regulations, such as nutrient sensing 5-AMP-activated protein kinase (AMPK) and mTOR pathways. Metformin targeting AMPK could attenuate the production of proinflammatory SASP, and rapamycin could inhibit mTOR pathway slowing the aging process both in vitro and in vivo (Imai and Guarente 2014; Mitchell et al. 2014; Mercken et al. 2014). Sirtuins play an essential role in many processes, especially Sirt1 and Sirt6 that are closely related to life span and health span (Fontana and Partridge 2015; Mitchell et al. 2014; Mercken et al. 2014; Field and Adams 2017; Selman et al. 2009). Animal studies showed individual sirtuin family members have dramatic effects on chromatin regulation, and the loss of function would cause progeria and a series of aging-related diseases (McGuinness et al. 2011).

It is also a promising strategy to target the cellular methylome. It has been proved by several studies that there is a functional relationship between differential DNA methylation, transcriptomic effects, and the development of aging-related renal diseases. The synergistic removal of calciprotein particles may be a good strategy to attenuate the adverse effects of hyperphosphatemia on the epigenome (Painter et al. 2008; Au et al. 2013). As mentioned before, Klotho promoter methylation is a feature of chronic kidney disease, which is usually found in elderly individuals. Treatment with Rhein reverses Klotho methylation efficiently in murine models (Selman et al. 2009).

More interventions deserve further investigation, like activation of telomerase and reduction of p16INK4a, which is potentially functional but also with adverse effects. Stains delay senescence of endothelial cells through reducing overproduction of intracellular OFRs and inhibiting nuclear export of telomerase reverse transcriptase (Haendeler et al. 2004). PPAR-g agonists protect against renal injury in

aging by reducing proteinuria, improving GFR, and decreasing sclerosis. On one hand, PPAR-g agonists could regulate p66SHC phosphorylation, which is an integration point for many signaling pathways that influence mitochondrial function and longevity. On the other hand, PPAR agonists could also increase expression of Klotho and decrease systemic and renal oxidative stress and regulate pathways that are associated with cell senescence in the kidney (Yang et al. 2009).

It is deniable that the epigenetics of aging is clearly important for health span. However, it still needs to be clarified whether the effect is uniform among different organisms. Furthermore, regulation of epigenetics of aging has different meanings for life course in the early stage and late stage, which suggests that aging-associated epigenetic processes might be subject to context-dependent intervention.

11.3.3 Caloric Restriction

Long time ago, McCay and colleagues have demonstrated caloric restriction to be able to extend life span of rats. Since then, calorie restriction has consistently shown beneficial effects on longevity, age-associated diseases, tumorigenesis, and attenuation of functional decline across many kinds of organisms (Gross and Dreyfuss 1984; Kritchevsky 2002; Weindruch and Sohal 1997). However, long-time caloric restriction is not feasible for humans, but short-time calorie restriction could produce significant reductions in body weight, blood pressure, blood cholesterol, and blood sugar, as well as reduced the development of atherosclerosis and ameliorated the decline in diastolic function (Walford et al. 2002). The exact mechanism of calorie restriction is still unclear. There are several extensive mechanisms that have been found, including reducing insulin and insulin-like growth factor 1 (IGF-1) signaling, reduction in oxidative stress, increase of antioxidant (De Cabo et al. 2004), enhanced mitochondrial function (Nisoli et al. 2005), improved proteostasis and autophagy (Cuervo 2008; Hansen et al. 2008), and a reduction in reproductive investment (Mitchell et al. 2015).

It has been shown that caloric restriction in animals modulates the aging-related physiological processes in the kidney. Caloric restriction could increase the resistance of the rat kidney to ischemic injury, since kidneys are usually susceptible in elderly individuals. The mechanism might rely on the molecular alterations such as the attenuation of aging-related changes in expression of genes such as claudin-7, kidney injury molecule-1, and matrix metalloproteinase-7 (Chen et al. 2007). Furthermore, it has been found that calorie restriction could reduce glomerulosclerosis, tubular atrophy, interstitial fibrosis, vascular wall thickening, and the expression of cytochrome c oxidase-deficient tubular epithelial cells (McKiernan et al. 2007). Calorie restriction could also prevent or delay the development of structural and functional changes, such as glomerulosclerosis and tubulointerstitial damage (Keenan et al. 2000). As for 24-month-old rats, calorie restriction could reduce the aging-related proteinuria, extracellular matrix accumulation, and the renal expression of connective tissue growth factor, vascular endothelial growth factor, and plasminogen activator inhibitor-1. The authors concluded caloric restriction affects

aging-related renal physiology by altering renal SREBP expression and renal lipid accumulation, since those changes of the kidney are associated with a decline in the renal expression of sterol regulatory element-binding proteins, SREBP-1 and SREBP-2, and a decline in renal triglyceride and cholesterol content (Jiang et al. 2005). The markers of oxidative stress, malondialdehyde and 4-hydroxynonenal, and markers of apoptosis, Bax and procaspase 3, were significantly lower in aged rats under calorie restriction compared to the rats fed ad libitum (Lee et al. 2004). Calorie restriction has been found to be an effective way to activate Sirt1, which has been proved as a protective factor of the kidney as mentioned before.

11.3.4 Renal Transplantation

Studies have clearly shown that the elderly patients with end-stage renal failure could benefit from kidney transplantation (Silva 2005b; Macrae et al. 2005). However, the survival of renal transplant recipients is still lower than that of the general population. It is not surprising that among the more than 300,000 US patients currently on dialysis, the patients with end-stage kidney disease are popular, especially around 70–80 years old (Silva 2005b). Questions come like whether a donor kidney should be transplanted to an elderly patient with comorbidities and an average life expectancy of 10–15 years over a younger patient. Studies showed the relative risk of renal graft failure, adjusted for comorbidities, is statistically similar for renal transplant recipients older than 65 years and their younger counterparts (Fabrizii and Horl 2001). The most common cause of graft loss in the elderly transplant recipient is the comorbidity-related demise of the patient. Thus, careful screening of elderly disease, peripheral vascular disease, diabetes mellitus, and chronic obstructive pulmonary disease may help minimize early posttransplant morbidity and mortality (Zhou et al. 2008a). Older renal allografts do have an increased risk of graft failure, mainly because of aging-related decline in renal function, predisposition to ischemia and drug toxicity, reduced capacity for repair, and a higher degree of immunogenicity (Nyberg et al. 2005; Bunnapradist et al. 2003).

Renal transplantation seems to halt but not reverse vascular calcification (Cianciolo et al. 2014). Exercise capacity and muscle strength were lower in renal transplant recipients than in healthy controls, but improved significantly following a rehabilitation program, indicating an important reversible component (van den Ham et al. 2005, 2007). With regard to the underlying mechanisms of premature aging, oxidative stress generally improves or even normalizes after renal transplantation. Levels of advanced glycation and products, which are associated with aging, decrease after kidney transplantation (Jin 2010; Crowley et al. 2013). Immunosuppressive treatment was reported to affect renal transplantation. Treatment of rapamycin could lower serum phosphate levels and increase insulin resistance in renal transplant recipients (Tataranni et al. 2011). Rapamycin treatment also increased Klotho expression in immortalized proximal tubular cell lines, consistent with the supposed antiaging properties (Tataranni et al. 2011). Another example of the complex effects of immunosuppressive agents concerns the reduced telomerase

activity associated with use of azathioprine (Getliffe et al. 2005). Corticosteroid treatment after transplantation might accelerate senescent processes (Bauer et al. 2009).

11.4 Conclusion

Aging is accompanied with decreased function of all organs, including the kidney. Old kidneys are functional but fragile. Although much progress has been made in the understanding of the renal aging process and the associated decline in renal function, there are still some questions remaining unanswered and deserving future investigation. Improved understanding of renal aging may help to optimize management of renal allografts obtained from older donors. More research should be done on the distinction between the decline in renal function and alteration in renal structure because of aging-associated diseases such as hypertension and diabetes mellitus and that solely because of aging.

As mentioned before, there are several ways to interfere the process of renal aging, but they are not working efficiently enough separately. Combination of different ways could be a potential method to prevent renal aging or attenuate aging and aging-related diseases. The final goal of any intervention battling features of kidney aging is to preserve long-term kidney health and function.

The epigenetics offers the promise of providing a context-dependent understanding of human health span. Improved knowledge of how epigenetic process regulates aging and how they interact with disease process will enable the identification and stratification of patients at increased risk of age-related morbidities. How to identify and track several of environmental factors influencing health and interacting with individual cellular and molecular processes is critical to the identification and curing for renal diseases. Long-term calorie restriction with optimal nutrition is pretty hard to achieve in daily life, which makes it unlikely to become clinically relevant in the near future. Some researches come up with several nutritional factors and small compounds mimicking the calorie restriction effects by regulating nutrient sensing pathways or by inducing autophagy are in different phases of preclinical and clinical testing.

All in all, epidemiologic, biochemical, and molecular evidence suggest that aging of the kidney is a complex interplay of different molecular mechanisms going far beyond a simple “wear and tear” process. There are many pathways found to be useful targets for slowing the aging process. Because combatting aging will require a long-term strategy, the route to clinical translation requires further insight from preclinical studies.

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Aging of the Bone

12

Yu Wei and Yao Sun

Abstract

Besides mechanical and protective function, bone serves as a keeper for marrow cells and an organ for regulation of calcium ion homeostasis. During aging, significant amounts of the bone are lost due to the loss of this delicate balance toward increased bone resorption coupled with decreased formation, which leads to net bone loss of the aging people. Osteoblasts, osteoclasts, and osteocytes are defined by their respective functions of bone formation and bone resorption. So, during bone aging, how the bone and bone cells will change are key issues for understanding osteoporosis. In this chapter, we focus on the changes of these factors during aging of the bone.

Keywords

Bone · Osteoblasts · Osteoclasts · Osteocytes · Osteoporosis

Bone loss caused by aging is the major reason of senile bone diseases such as osteoporosis and osteoporotic fractures (Chen et al. 2014). Osteoporosis is an enormous public health problem that will only increase in scope with aging. Prevalence of osteoporosis in the United States is estimated to increase to >14 million people in 2020. Hip/femur and vertebral fractures are the most common sites (de Waure et al. 2014). Osteoporosis causes more than 2 million fractures annually (Gambacciani et al. 2013).

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12.1 Aging of Bone

Osteoporosis is a common disease that manifests itself as fractures occurring at multiple skeletal sites, such as the spine, hip, or wrist, and causes significant morbidity and mortality. Aging-related changes of bone cells, extracellular matrix, and key molecules involved are factors that cause both bone loss and bone fractures.

Osteoporosis is characterized by lower bone mass and an increased risk of fracture. During aging, due to the tipping of this delicate balance toward enhanced resorption, significant amounts of the bone are lost coupled with decreased formation. The diagnosis of osteoporosis relies on bone mass density measurement by means of T-score that is defined as the quantity of standard deviations (SDs) from the average BMD of the healthy young. Osteoporosis is diagnosed if T-score is ≤ -2.5 SD according to the World Health Organization (WHO) (de Waure et al. 2014).

12.2 Cell Types in the Bone

Skeletal component cells include osteoblasts, osteoclasts, osteocytes, chondrocytes, and their progenitor cells, bone marrow stem cells (BMSCs). They play roles in skeletal development and maintenance, as well as in the pathogenesis of osteoporosis. Osteocytes are the most abundant cell type in the bone. Osteoblasts and osteoclasts (Fig. 12.1), existing on bone surface, are defined by their respective functions of bone formation or bone resorption. Both osteoblasts and osteoclasts function in

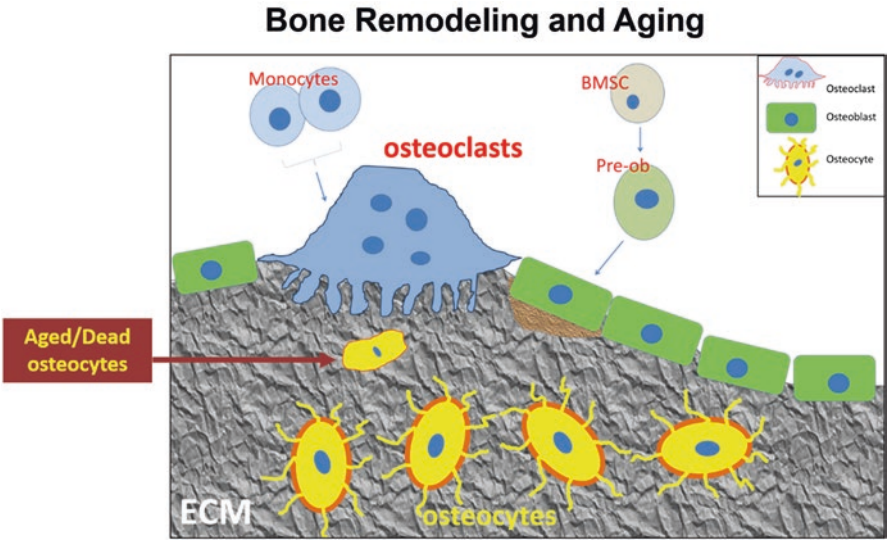

Fig. 12.1 Bone cells and bone aging

concert, which requires intimate cross talk with osteocytes. Senescence of cells in the bone leads to loss of the bone and osteoporosis in aged people.

12.2.1 Osteocyte Aging

In contrast to osteoblasts, which were of only a small fraction of the bone, osteocytes were long-lived and far more abundant than osteoblasts (Manolagas and Parfitt 2010). Osteocytes comprise more than 90% of all cells in the adult bone. Osteocytes are embedded within the mineralized bone matrix. Osteocytes remained connected to one another via a network of cell projections (Bonewald 2011). Osteocytes can live for decades, whereas osteoblasts live only for several months. It has become evident that osteocytes were the choreographers in the remodeling process of the bone (Manolagas and Parfitt 2010; Nakashima and Takayanagi 2011). Because of their longevity, osteocytes are more likely than osteoblasts to accumulate molecular damage over time (Jilka and O'Brien 2016). Osteocytes can undergo apoptosis, which may have specific regulatory effects on bone resorption and remodeling (Chen et al. 2015).

Osteocytes secrete proteins related to mineralization and phosphate metabolism, including bone sialoprotein (BSP), dentin matrix protein 1 (DMP1), matrix extracellular phosphoglycoprotein (MEPE), phosphate-regulating gene with homologies to endopeptidases on the X chromosome (PHEX), and fibroblast growth factor 23 (FGF23) (Dallas et al. 2013; Ubaidus et al. 2009). Expression of the transmembrane glycoprotein E11/gp38 and membrane type 1 matrix metalloproteinase (MT1-MMP) is also required for the formation of osteocyte dendritic processes and canaliculi (Holmbeck 2005). Osteocytes also secrete proteins that affect bone formation, including Dickkopf-related protein 1 (Dkk1) and sclerostin, which are primarily expressed in osteocytes.

During aging, osteocytes showed degenerative changes. Osteocyte death occurs during aging, especially after menopause. Osteocyte cell population and lacunar density were expected to decrease. In addition, Milovanovic et al. reported that deterioration of the canalicular network with age reduces connectivity between osteocytes and other tissues. The structure of the osteocyte network also changes with age in both mice and humans (Jilka and O'Brien 2016). Since osteocytes can sense matrix strain directly via their cell bodies and the magnitude of the osteocyte signal is likely related to the number of osteocytes and their canaliculi contributing to the signal, the variations in osteocyte morphology and osteocyte number density may cause changes in mechanotransduction (Hemmatian et al. 2017). Due to an increase of pro-apoptotic systemic and mechanical factors, the osteocyte lacunae are becoming smaller with aging (Hunter and Agnew 2016). Reduced osteocyte density with age is due to decreases in physical activity stimulation, reduction of the mechanical loading with sports, increases in endogenous glucocorticoids, and accumulation of reactive oxygen damage in the bone (Chen et al. 2015).

Apoptotic osteocytes release factors that instruct neighboring active osteocytes to produce receptor activator of nuclear factor ΚB ligand (RANKL). RANKL is one of the key molecules essential for osteoclast formation and activation. RANKL interacts with its receptor RANK, which is expressed by osteoclasts. As a decoy receptor, osteoprotegerin (OPG) is believed to function primarily in modulating interactions between RANKL and RANK. The local increase of osteoclasts associated with apoptotic osteocyte is the result of loss of OPG-producing osteocytes and increased production of RANKL by neighboring viable osteocytes. In addition, osteocyte apoptosis is predicted to enhance bone resorption through the release of ATP, and the subsequent osteocyte necrosis is predicted to enhance bone resorption through the release of DAMPs, leading to the replacement of damaged bone (Komori 2016). The microdamage of the bone are also associated with osteocyte apoptosis and targeted bone resorption. Another widely accepted cause of cellular and macromolecular damage in aged cells is the increased levels of ROS, NOS, and other oxidants due to dysregulated cellular respiration (Lopez-Otin et al. 2013).

Autophagy was a major physiological process in which dysfunctional cytosolic macromolecules, membranes, and organelles were targeted and delivered to lysosomes for degradation and recycling (Chen et al. 2014). Recently, autophagy gained great interest due to its role in age-related and degenerative diseases. Thus, autophagy is important for the health and viability of long-lived cell types (Onal et al. 2013). Besides, changes in osteocyte autophagy over time could contribute to skeletal aging. Decline of osteocyte autophage with aging promotes cell death pathways such as apoptosis (Jilka and O'Brien 2016). Decreased activity of osteocyte autophagy might have a contribution to the age-related bone loss in aging osteoporosis (Chen et al. 2014).

12.2.2 Osteoblast Aging

Osteoblasts are the main functional cells of bone formation. They are responsible for the synthesis, secretion, and mineralization of bone matrix. Between 60% and 80% of the osteoblasts that originally assemble at the resorption site have been estimated to die by apoptosis. With accelerated aging, osteoblasts exhibited impaired cell activity and reduced differentiation capacity. Also, senescent osteoblasts have significantly impaired mineralization capacity: the potentials of osteoblasts to differentiate toward mature osteoblasts or express and secrete collagen type 1 (Col1) were significantly decreased. Also, senescent osteoblasts remarkably secrete less osteocalcin and C1CP, a collagen synthesis marker, and are less responsive to stimuli of growth factors such as insulin and IGF-I.

Furthermore, aging of the bone has been partly attributed to apoptosis of osteoblasts and osteocytes. Parathyroid hormone (PTH) and calcitonin exert anabolic effects on the bone by inhibiting osteoblast and osteocyte apoptosis (Stanislaus et al. 2000; Plotkin et al. 1999). Recent study analysis of senescent osteoblasts revealed that they failed to mineralize bone matrix and increased bone resorption. Tartrate-resistant acid phosphatase (TRAP) staining revealed that nonsenescent

osteoblasts induced very few osteoclasts, while senescent osteoblasts stimulated osteoclast formation. This process is driven by secretory phenotype factor, IL-6. Neutralization of IL-6 was sufficient to inhibit senescence-induced osteoclastogenesis (Luo et al. 2016). Parathyroid hormone stimulation of cAMP accumulation in osteoblasts decreases with aging. Osteoblasts from aged animals also respond poorly to fluid flow and flow-induced intracellular calcium oscillations (Ota et al. 2013). Expression of the nuclear receptor Ror-β in osteoblast precursors is elevated in cells from aged mice compared to young mice. In addition, senescent osteoblasts increase tumor cell seeding and metastatic tumor burden in the bone.

12.2.3 Osteoclast Aging

Skeletal aging is characterized as an excess of bone resorption. The osteoclast is one of the components of bone tissue and performs the function of bone resorption. Osteoclasts are large, multinucleated cells derived from the nonadherent hematopoietic stem cells present in the bone marrow. They are short-lived cells (2–4 weeks). The osteoclast markers include Ctsk, Oc-stamp, Oscar, Tnfrs11a (Rank), and Acp5 (Trap). There are age-dependent changes in the expression of osteoclast differentiation factors and receptors, such as RANKL/RANK/OPG and M-CSF pathways in human marrow cells (Chung et al. 2014). Osteoclasts from aged mice also produce sclerostin that may contribute to the bone formation impairment in aged bone (Chung et al. 2014). Among the major bone cell types, osteoclasts require very low levels of ROS for differentiation and function. Loss of caspase-2 enhances resistance to oxidants, as measured by decreases of the oxidative stress-induced apoptosis of osteoclasts. Caspase-2 maintains bone homeostasis by inducing apoptosis of oxidatively damaged osteoclasts (Sharma et al. 2014).

12.2.4 Mesenchymal Stem Cell Aging

The bone marrow microenvironment is a complex environment constituted by stromal cells, the extracellular matrix, and a variety of soluble cytokines, which provides the foundation for stem cell survival. There are two key types of stem cells, hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), with the potential of both self-renewal and multiple differentiations (Prockop 1997; Bruder et al. 1997). Bone marrow mesenchymal stem cells (BMSCs) are derived from the early development of mesoderm and ectoderm. BMSCs are one of the important members of stem cells family, which have pluripotent ability. MSCs have the ability to self-renew and differentiate into multiple lineages, including osteogenic, adipogenic, and chondrogenic (Prockop 1997; Bruder et al. 1997). By aging, the proliferative capacity of BMSCs was decreased, the number of osteoblasts was decresed and the adipogenesis of BMSCs was increased (Stenderup et al. 2003; Hitesh et al. 2016).

It is known that deficiency in number and function of osteoblasts, together with increase of marrow adipogenesis, accounts for the key etiological factor of osteoporosis. BMSCs in the marrow pool are the major source of adipocytes. With bone aging, the BMSC-derived fat cells accumulate in the marrow. Unbalanced differentiation of BMSCs into marrow adipocytes and osteoblasts can result in bone loss by the excessive accumulation of marrow adipocytes. PPARγ is upregulated during aging, and it is involved in adipocyte differentiation in vitro and in vivo as a key transcription factor. PPARγ can induce the differentiation of BMSCs into adipocyte lineage. Besides, it can negatively regulate osteoblast differentiation by means of suppressing expression of osteoblast-specific transcription factors.

12.3 Aging of Cartilage

For millions of aged people worldwide, osteoarthritis (OA) is the most common chronic joint disease. OA incidence increases with age, and over 75% of the population displays radiographic evidence of the disease after 60 years old. Articular cartilage is a conjunctive tissue composed of only one cell type, chondrocytes. Chondrocytes are enclosed in a self-synthetized extracellular matrix (ECM). They are responsible for matrix composition and integrity, thereby conferring to cartilage its functions of mechanical support and joint lubrication (Archer et al. 2003). Compared to normal cartilage, the formation of chondrocytes, presence of irregular cartilage surfaces, loss of cartilage volume, and matrix calcification are shown in OA cartilage (Speziali et al. 2015). These changes in cartilage are linked to the alteration of molecular components of cartilage ECM. Typically, the decrease in proteoglycan becomes prominent with disease progression. Breakdown of these components is handled by a set of aggrecanases and collagenases, which are upregulated during OA. Proinflammatory cytokines, such as IL-1β, TNF-α, and IL-6, are associated with OA occurrence and participate in cartilage degradation through activation of pathways (e.g., nuclear factor-κB, toll-like receptor) (Goldring et al. 2012).

12.4 Treatment of Osteoporosis

For treatment methods of osteoporosis, dietary sources of calcium and vitamin D are ideal. Pharmacological supplements can be used, if diet alone cannot satisfy the daily need. Bisphosphonates are first-line therapy drugs for patients with osteoporosis or at a high risk of osteoporotic fracture (Gambacciani and Levancini 2014). And for postmenopausal women who are at high risk of osteoporosis and fractures, denosumab, the monoclonal antibody to RANKL, can result in sustained reduction of bone turnover, continued gains in BMD, and continued low yearly fracture incidence (Papapoulos et al. 2015). Among the several types of therapeutic intervention in osteoporosis, hormone replacement therapy (HRT) has traditionally been seen as the gold standard method of preventing osteoporotic fractures among postmenopausal women (Gambacciani and Vacca 2004). For women who cannot be treated

with hormones, replacement of HRT is needed. Teriparatide (PTH) is the only available bone anabolic drug in clinic. However, it is not classified as a first-line drug. Selective estrogen receptor modulators (SERMs) are compounds that lack the steroid structure of estrogens but interact with estrogen receptors (ERs) as antagonists depending on the target tissue, such as raloxifene and bazedoxifene (Gambacciani et al. 2013). Although various medications have been proved effective in treating osteoporosis, there are also some potential hazards that should be taken in consideration, such as hypocalcemia, worsening of renal impairment, and osteonecrosis of the jaw (Sanders and Geraci 2013).

There is a growing evidence showing that one maintenance during early adult life is an important contributor to bone strength during aging. Insulin-like growth factor 1 (IGF-1), the most abundant growth factor in bone matrix, regulates bone mass in adulthood. Therefore, modulation of IGF-1 deposition in the bone matrix could potentially be a therapeutic approach to delay or prevent osteoporosis (Xian et al. 2012). Except traditional therapies, recently, it was reported that targeting senescent cells can reduce bone resorption and augment bone formation. In addition, senescence-associated secretory phenotype (SASP), developed by senescent cells, also have deleterious paracrine and systemic effects. It is found that AP20187 treatment, a drug which cannot only extend health span but also prevent the development of age-related diseases, resulted in obviously lower senescence biomarker p16Ink4a mRNA expression (by −59%) in the bone of mice. Furthermore, AP20187 treatments also resulted in an improvement of the osteoblast/osteoclast ratio in the experimental mice, resulting in lower bone resorption. Additionally, on the bone surface, osteoblast numbers, mineral apposition rate, and bone formation rate were all higher in the AP20187-treated mice than in the control ones, which suggest that decrease of senescence cells can augment bone formation (Farr et al. 2017).

There is also another therapy method for the elderly suffered with bone loss: exercise. For individuals with osteoporosis, they are recommended to engage in a multicomponent exercise program that includes resistance training as well as balance training, instead of engaging in aerobic training. For individuals with osteoporotic vertebral fracture, they are recommended to take safe and appropriate exercise, just like osteoporotics (Giangregorio et al. 2014).

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Ovarian Aging and Osteoporosis

13

Liyuan Li and Zhao Wang

Abstract

Osteoporosis is the most common bone metabolic disease with a very high morbidity, and women usually got a higher risk of osteoporosis than men. The high incidence rate of osteoporosis in women was mainly caused by (1) women having fewer skeletons and bone mass, (2) pregnancy consuming a large amount of calcium and other nutrients, and most importantly (3) the cease of estrogen secretion by ovaries after menopause. Along with ovarian aging, the follicle pool gradually declines and the oocyte quality reduced, accompanied with decline in serum estrogen. Estrogen deficiency plays an important role in the pathogenesis of postmenopausal osteoporosis; it is mainly a result of the recognition that estrogen regulates bone remodeling by modulating the production of cytokines and growth factors from bone marrow and bone cells. This review will summarize current knowledge concerning ovarian aging and postmenopause osteoporosis and also discuss clinical treatment and new ideas of drug development for osteoporosis.

Keywords

Ovary aging · Menopause · Osteoporosis

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13.1 Introduction

Ovaries are grape-shaped reproductive organs of female animals. The function of ovaries is to produce oocytes periodically and to secrete steroid hormones. The size of the ovaries is associated with female age and the spawning period. Oocytes grow and develop within the environment provided by ovarian follicles, which are composed of different number and types of cells according to the stage of folliculogenesis (Gougeon 1996). The follicular development process can be divided into three stages: primordial follicles (PmF), growing follicles, and Graafian follicles (GF); according to the size and the structural differences, the growing follicles can be divided into primary follicles (PrF) and secondary follicles (SF). There are also some follicles that cannot ovulate, which were called atretic follicles (AF) (Eppig and O'Brien 1996). Folliculogenesis ends when the ovaries are not capable of responding to hormonal cues that recruit follicles to mature, which are the signals of the beginning of menopause. Menopause is the final step in the process of ovarian aging (Broekmans et al. 2009).

The average age of menopause in women is approximately 51 years, resulting in a postreproductive period that extends for nearly one third of their lives (Treloar 1981). As a result, the mechanisms involved in the process of ovarian aging have gained increased attention and visibility. The study of ovarian aging was of high priority because of its effect on fertility and orthobiosis of women. With the sudden withdrawal of hormonal support associated with menopause, a number of physiological systems are affected, including bone density, cardiovascular health, and possibly some cancers (Gosden 1986; Prior 1998; Sherwin 2003). As a result, the study of ovarian aging and its related diseases was of great value to improve the quality of human life. In this chapter, we mainly discuss about ovarian aging and its associated disease osteoporosis.

13.2 Oocyte Development in a Lifetime

During fetal development, each woman receives an endowment of oocytes. At the 4th month of fetal development, ovaries contain about six to seven million oocytes surrounded by a flat granulosa cell layer to form the primordial follicle pool (Baker 1963; Block 1952, 1953). At any particular age, the majority (more than 99%) of oocytes in the ovary are present as nongrowing primordial follicles. Due to a rapid loss of the great majority of the primordial follicles via apoptosis in the second half of fetal life, only less than two million primordial follicles remain at birth (Markstrom et al. 2002). After birth, this high rate of follicle loss slows down somewhat, so that at menarche at least 300,000–400,000 primordial follicles remain (Block 1953; te Velde and Pearson 2002). During the reproductive years, the continued and gradually accelerated decline will cause numbers to have dropped below 1000 at the time of menopause (Faddy 2000; Faddy and Gosden 1996; Richardson et al. 1987).

Along with the decrease in follicle number, oocyte quality also diminishes. The reproductive aging process is thought to be dominated by a gradual decrease in both

the quantity and the quality of the oocytes residing within the follicles present in the ovarian cortex (Broekmans et al. 2009) (te Velde and Pearson 2002). Based on the studies that estimated the actual primordial follicle numbers (Block 1952; Gougeon and Chainy 1987; Jansen 1993; Richardson et al. 1987), it was concluded that the rate of oocyte decline follows a biphasic pattern, with a distinct acceleration at about age 38 as women age toward menopause (Broekmans et al. 2009).

The loss of oocyte quality is believed to be due to the increase in meiotic nondisjunction, resulting in an increasing rate of aneuploidy in the early embryo at higher female ages (Battaglia et al. 1996; Hunt and Hassold 2008; Kuliev et al. 2005; Pellestor et al. 2005). Underlying mechanisms may involve accumulated damage of oocytes in the course of a woman's life or age-related changes in the quality of the granulosa cells surrounding the oocyte.

13.3 Hormones Produced by Ovaries

Ovary is capable of secreting steroid hormones, estrogen and progesterone, and a small amount of testosterone and inhibin. Progesterone is an endogenous steroid; it functions with estrogen through promoting menstrual cycle changes in the endometrium. Progesterone is also involved in pregnancy and embryogenesis of humans and other species. In women, about 55% of testosterone was produced by the ovaries and adrenal glands and released to the bloodstream directly.

Estrogen has an important physiological role; it is a substance that promotes the secondary sexual development and sexual maturation in female animals. Besides, estrogen has a significant impact on maintaining the homeostasis of the endocrine system, cardiovascular system, and metabolic system and bone development.

The bioactive estrogen produced by ovary is mainly estradiol, which was synthesized by granule cells in ovaries (Tsuchiya et al. 2005). The process is to convert androstenedione into estrogen: in the action of LH, endometrium cells first turn cholesterol into androstenedione; then under the function of FSH, granule cells produce aromatases to convert androstenedione into estrogen. The converted estrogen will be secreted into follicle fluid and blood. The blood estrogen will be activated and transformed to estrone and estriol in the liver and finally combine with glucuronic acid or sulfuric acid to excrete with urine.

Menopause is characterized by a marked reduction in estrogen production as the main site of synthesis changes from the ovaries to peripheral tissues. Whereas in premenopausal women production rates for estradiol and estrone are of the order of 400 μg/24 h and 180 μg/24 h, after menopause, they decrease to 6 μg/24 h and 40 μg/24 h, respectively (Gray and James 1979).

13.4 The Constitution and Function of the Bone

Skeletal system was consisted of many individual bones and their associated connective tissues. In our body, the main function of bone is to provide mechanical support and to supply minerals during metabolism. Bone is consisted of organic protein matrix and inorganic calcium phosphate, which is mainly composed of hydroxyapatite (HA) (Kartsogiannis and Ng 2004; Tracy and Doremus 1984). With the presence of minerals, bone tissue is much harder compared with other tissues in our body. The skeletal system has many biomechanical functions, such as maintaining body shape; protecting various organs in the cranial cavity, thoracic cavity, and pelvic cavity; protecting the bone marrow; and so on.

The adult bone consists of the cortical bone and cancellous bone, among which cancellous bone presents as a porous spongy mesh; it is mainly composed of trabecular spicule and trabecular sclerite. The cortical bone is the shell bone structure wrapped around the bone. Cortical bone is hard and dense; it is the main component of the skeletal system (Bronner and Worrell 1999).

The bone is mainly composed of inorganic minerals, organic materials, cells, and fats. Bone formation is a biomineralization process. Organic materials account for 20–40% of the bone; it is mainly composed of bone collagen type I, and small amount of collagen type III, collagen type V, and collagen type X (Recker 1983). The collagen units aggregate to form collagenous fibers, and collagenous fibers are arranged in order to form bone lamella. The main component of the bone mineral hydroxyapatite deposits in the spaces of collagen fibers by crystallization. The cross-linking of collagen fibers enhances the stability of bone lamella; the deposition of hydroxyapatite collagen fiber spaces greatly enhanced the hardness of the bone matrix.

There are some non-collagen proteins that also play a key role in bone matrix mineralization such as osteocalcin (OCN), osteopontin (OPN), and osteonectin (ON) (Mcclung 2016). The function of these proteins was to accurately direct the deposition of hydroxyapatite into collagen fiber spaces and to coordinate the balance of osteoblasts and osteoclasts. The bone turnover rate and the activity status of osteoblasts can be reflected by detecting the content of osteocalcin in serum.

13.5 Functional Cells in Bone

13.5.1 Osteoblasts

Osteoblasts are a group of cells arranged on the surface of the bone; they are mainly differentiated from mesenchymal stem cells (MSCs) from the internal or external periosteum or from the bone marrow. Under external stimulation, osteoblasts can differentiate into bone cells, bone lining cells, or apoptosis (Noble et al. 1997; Tomkinson et al. 1997). Osteoblasts play a key role in the process of collagen synthesis, secretion, and mineralization. Osteoblast contains abundant endoplasmic reticulum and Golgi apparatus, which were of benefit to the synthesis and secretion

of bone metabolism proteins such as osteocalcin and bone sialoprotein. The functional activity of osteoblasts can be detected by alkaline phosphatase (ALP) staining assay (Mcclung 2016).

Osteoblasts have receptors for many regulatory factors, such as the sex hormone receptor, bone morphogenetic protein (BMP) receptor, prostaglandin receptor, RANKL receptor, and so on. The external regulatory factors bind to their corresponding receptors on the surface of osteoblasts to activate or suppress intracellular signaling pathways, regulating the proliferation, differentiation, life span, and function of osteoblasts. Some regulatory factors also involved in regulating the balance between bone cells, for example, RANKL, can be synthesized by osteoblasts and inhibit the formation of osteoclasts (Udagawa et al. 1999).

The formation of the bone can be divided into two stages: (1) the formation of bone matrix and (2) the mineralization process. Osteoblasts synthesize and secrete collagen, collagen units orderly gather together to form collagen fibers, and collagen fibers arrange on the surface of the bone to form multilayer bone lamella; this organic matrix secreted by osteoblasts was called osteoid. With the ongoing bone matrix formation, osteoblast was buried inside osteoid gradually and then further differentiated into bone cells (Merz and Schenk 1970). Bone mineralization occurred after osteoid gets matured; mineral ions orderly and accurately deposit inside the spaces of collagen fibers under the guidance of non-collagen proteins to form hydroxyapatite crystals (Harris 1960).

13.5.2 Osteoclasts

Osteoclast is a large multicellular cell that is usually derived from mononuclear phagocytes from the bone marrow; the main function of osteoclast in the animal body is bone absorption. Similar to osteoblasts, the growth and differentiation of osteoclasts are regulated by many regulatory factors, including calcitonin, vitamin D3, parathyroid hormone, interleukin-1, interleukin-6, and so on.

After activation, osteoclasts migrate and attach to calcified bone surface. The side of osteoclast toward bone surface stretch out many small protuberances. Cytoplasm near protuberance side in osteoclast is rich in microfilaments; this special structure makes it possible that protuberances attach close to bone surface and form microenvironment between cell and bone surface. By releasing lysosomal enzymes and acids into microenvironment, inorganic minerals inside bone matrix were dissolved and swallowed by the osteoclasts through the protuberances area. Thus, the process of bone matrix decalcification (Cowin 2001) was completed. The inorganic minerals that entered osteoclast were degraded inside the cell and then released to the blood again in the form of mineral ions (Boyle et al. 2003).

13.5.3 Bone Lining Cells and Osteocytes

Both bone lining cells and bone cells were differentiated from osteoblasts. Bone lining cells are a layer of cells covering the inner surface of resting bone. Bone lining cell layer and the osteoid layer together formed the common barriers on bone surface, which prevented the inappropriate dissolution of bone by osteoclasts or inflammatory cells (Miller et al. 1980). Bone cell is the only functional cell buried inside bone matrix; differentiated bone cells are smaller in size and have less organelles compared with those of the precursor osteoblasts. Elongated synapses extended outward from bone cell edge, connecting with synapses of adjacent bone cells, bone lining cells, and even some osteoblasts through gap junction. This gap junction finally forms a three-dimensional network inside bone tissue, which was also called bone sunken-bone canalicule network. Outside mechanical signals applied on animal body can be transformed into biological signals through this three-dimensional network. Signals transmit to the surface of the bone via the gap junction of synapses and initiate the activation of target cells.

13.6 Imbalance of Bone Metabolism and Related Bone Diseases

In the last section, we mentioned that osteoblasts and osteoclasts correspond with each other and play an important role in bone development. The reconstitution of the bone continually proceeded during a person's whole life, realizing through old bone resorption by osteoclasts and new bone formation by osteoblasts (Manolagas and Jilka 1995). The self-renewal and reconstitution of bone tissue was accomplished through bone reconstructive unit (BRU) based on osteoblast-osteoclast coupling. When the bone remodeling unit is activated, BRU begins to absorb old bones through osteoclast resorption, and then osteoblasts function to form new bones and complete bone turnover for one time. The activation efficiency was defined as the number of times BRU appeared per unit time. Through this constantly self-renewal capacity, the skeleton system adapts to external environmental changes caused by both mechanical and nonmechanical factors.

New bone formation and old bone absorption maintain a dynamic equilibrium status at normal situation. However, the destruction of this balance can easily trigger bone metabolic disease. Bone metabolic diseases include abnormality in bone growth, bone absorption, and mineral deposits, with the corresponding clinical manifestations of osteoporosis, osteomalacia, or osteopetrosis. Of all the bone metabolic diseases, osteoporosis has the highest morbidity, which greatly affects the quality of human life.

The pathogenesis of osteoporosis is mainly due to the new bone formation which does not fill the gap left by the absorption of the old bone, which results in a negatively balanced bone metabolism. When the bone reconstructive unit was negatively activated, the higher the activation rate and the stronger the bone turnover, the greater is the bone loss (Manolagas and Jilka 1995). This is what happens when

serum estrogen declines. Osteoporosis is characterized by reduced bone mineral density, bone microstructure degradation, trabecular bone loss, and increased risk of fracture (Golob and Laya 2015). Compared with normal people, patients with osteoporosis often have a morbid humpback or excessive bending legs in appearance. The World Health Organization survey shows that more than 200 million people are currently suffering from osteoporosis, and the incidence rate of osteoporosis is still increasing year by year (Page 2010). The 2013 urban residents' health survey of China showed that osteoporosis has become one of the common chronic diseases among urban residents, following other metabolic diseases like high blood pressure, diabetes, and obesity, which all need the extensive attention from the whole society.

13.7 Estrogen and Bone Metabolism

Estrogen was important to bone metabolism in human body. In the year 1941, Albright found that the pathogenetic process of osteoporosis accelerated immediately following both the natural and unnatural menopause (Albright et al. 1941), thus putting forward the hypothesis that osteoporosis was related to the decrease in ovarian function. In the year 1969, Riggs demonstrated that bone turnover accelerated during perimenopausal and early menopause period (Riggs et al. 1969). Estrogen replacement therapy can prevent bone loss caused by menopause (Vedi and Compston 1996).

Both osteoblast and osteoclast have estrogen receptors (Eriksen et al. 1988); estrogen interacts with its receptor through both traditional and nontraditional pathways. In the traditional pathway, estrogen bonds to homologous estrogen receptor in the nucleus; the compound then regulates gene transcription directly or combines with translational factors. In the nontraditional pathway, estrogen binds to membrane receptors and mainly regulates the apoptosis process of osteoclast, osteoblast, and osteocytes.

Estrogen plays different roles in the skeleton on organ level, tissue level, and cellular level. On organ level, the role of estrogen is to maintain bone substance. On tissue level, estrogen inhibits bone transformation and maintains the balance between osteogenesis and bone resorption. On cellular level, estrogen affects the formation, the biological cycle, and the functional activity of both osteoclasts and osteoblasts. Estrogen affects bone metabolism mainly through the following mechanisms:

1. Estrogen stimulates bone formation through direct effect on osteoblasts.

Studies of long-term estrogen replacement therapy (ERT) in high doses have demonstrated that estrogen increased osteoblast activity (Vedi and Compston 1996; Wahab et al. 1997). In vitro culture of pure rat osteoblast-like cells with 17β-estradiol treatment significantly enhanced cell proliferation and the expression of collagen gene (Ernst and Froesch, 1988). Estrogen regulates a dual differentiation process by

promoting early osteoblast differentiation and inhibiting adipocyte differentiation in mouse bone marrow stromal cell line (Okazaki et al. 2002). The regulation of estrogen on human osteoblast is determined both by the differentiation and ER isoform expression (Waters et al. 2001).

2. Estrogen influences the formation of osteoclasts by regulating the level of cytokines and growth factors.

Osteoblast is capable of expressing and secreting a series of cytokines or growth factor that regulate the formation of osteoclast. RANKL (receptor activator of NF-κB ligand) is a membrane-bound molecule that has been identified as a member of tumor necrosis factor (TNF) ligand family. RANKL is essential for osteoclast formation; in vitro culture of bone marrow macrophages (BMMs) with the addition of RANKL leads to osteoclast formation (Chen et al. 2007). There are two receptors for RANKL: (1) RANK, a membrane receptor expressed on cell surface of osteoclast progenitors, and (2) osteoprotegerin (OPG), a secreted cytokine receptor. OPG acts as a decoy receptor through blocking the combination of RANKL with the functional receptor RANK, thus inhibiting osteoclastogenesis (Fig. 13.1).

IL-1 is a strong cytokine that can induce bone erosion through activation of osteoclasts in inflammatory site such as in rheumatoid joint regions. IL-1 is not only capable of activating osteoclasts but is also involved in the multinucleation, differentiation, and survival of osteoclasts. IL-1 alone is not capable of inducing the differentiation of osteoclasts from precursor cells (BMMs); it acts as a synergistic effect on RANKL-induced osteoclast formation (Kim et al. 2009). Other cytokines secreted by osteoblast included IL-6, TNF, and so on, which also participate in osteoclast formation (Fig. 13.1).

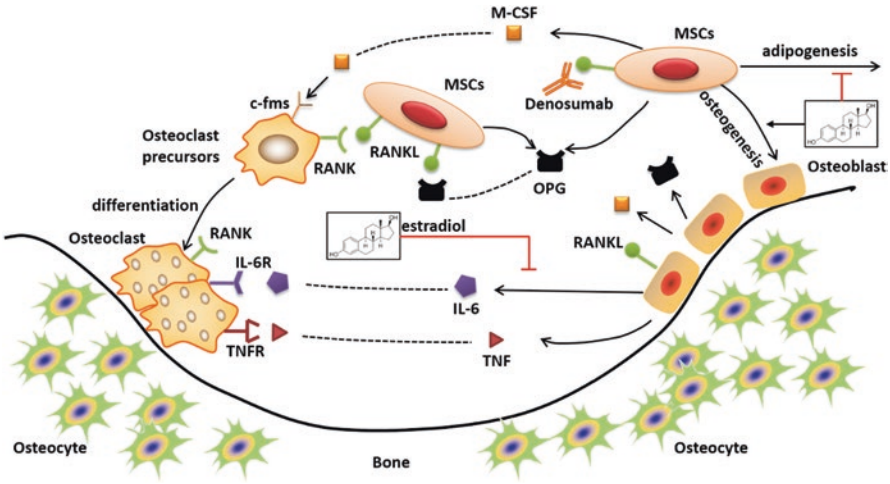

Fig. 13.1 Interactions between osteoblast and osteoclast. Mesenchymal stem cells (MSCs) and osteoblast are capable of secreting a series of cytokines to regulate osteoclastogenesis

3. Estrogen modulates osteoclast lysosomal enzyme secretion, promotes apoptosis in osteoclasts, and inhibits apoptosis in osteoblasts and osteocytes.

Osteoclasts attach to bone surface to form a sealed extracellular compartment; an acid environment was formed through the action of a hydrogen pump in osteoclasts, into which osteoclast produces and secrets lysosomal proteases (Kremer et al. 1995). Studies have shown that estrogen directly acts on mature osteoclast to prevent the secretion of lysosomal enzymes by decreasing the secretion of cathepsin B, cathepsin L, and TRAP. On the other hand, estrogen also decreases the ability of osteoclast to form cavities between the cell and bone surface.

Another research conducted by Boyce showed that in vivo treatment of 17 beta estradiol increased osteoclast apoptosis and prevented ovariectomy-induced bone loss caused by ovariectomy. In vitro culture of bone marrow cells with 17 beta estradiol caused two- to threefold increase in osteoclast apoptosis during osteoclastogenesis (Boyce et al. 1996), indicating that estrogen promotes apoptosis in osteoclasts. On the contrary, researchers found that estrogen treatment reduced annexin V staining and DNA fragmentation in osteoblastic cells and increased heat shock protein 27 expression (Cooper et al. 2000), which has been demonstrated to reduce apoptotic cell death (Mendelsohn et al. 1991; Samali and Cotter 1996).

4. Estrogen affects bone metabolism by regulating the expression of different hormones.

Estrogen promotes calcitonin secretion and inhibits bone absorption, enhances the activity of hepatic 25-hydroxylase and renal 1 alpha-hydroxylase, increases the expression level of 1,25-(OH)2D3, and promotes intestinal calcium absorption. Calcitonin synthesis decreases when estrogen level decreases. Calcitonin inhibits osteoclast activity through combining to its receptor on osteoclasts, thereby reducing bone absorption (Fig. 13.2).

Secondly, estrogen can reduce the starting point that parathyroid hormone (PTH) senses to react to blood calcium, inhibit PTH secretion, and activate bone absorption process. In the deficiency of estrogen, the sensitivity of bone to PTH increases, and bone absorption is activated. Deficiency of estrogen can also influence adrenal cortical hormone, which leads to the delay of bone formation, reduction in intestinal calcium absorption, and calcium increase in both feces and urine.

The metabolism of estrogen occurs in the liver. Estradiol was first oxidized to estrone; under the catalysis of CYP450 enzyme (e.g., 2-hydroxylase and 16α-hydroxylase), estrone was hydroxylated to 2-hydroxyestrone or 16α-hydroxyestrone (Michnovicz and Rosenberg 1992; Niwa et al. 1990). 2-hydroxyestrone has less biological function. 16α-hydroxyestrone is antagonistic to estrogen in the skeleton, and it can inhibit bone transformation in ovariectomized rats (Westerlind et al. 1998). 16α-hydroxyestrone is also associated with the bone density of vertebrae and femur bone in postmenopausal women (Lim et al. 1997).

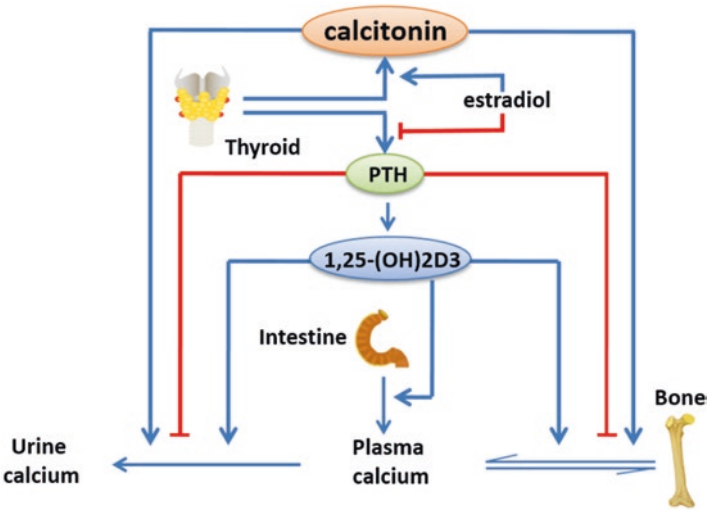

Fig. 13.2 The function of calcitonin and PTH in calcium metabolism

13.8 Clinical Treatment for Osteoporosis

Treatments for osteoporosis and its complications include non-drug therapy and drug therapy, as well as the use of bone replacement materials (Hench and Wilson 1984). Currently, there are two major categories of anti-osteoporosis drugs: anti-bone resorption drugs and anabolic drugs.

(a) Anti-bone resorption drugs

1. Bisphosphonates

Bisphosphonates are the most commonly used anti-bone absorption drugs for osteoporosis. Bisphosphonates have high affinity to hydroxyapatite, and they bind specifically to bone surfaces where bone remodeling is active. Bisphosphonates inhibit osteoclast function and thus suppress bone absorption. The bisphosphonates currently used in the prevention and treatment of osteoporosis include alendronate sodium, zoledronic acid, sodium risedronate, and so on. The main side effects of bisphosphonates include gastrointestinal reaction, hypocalcemia, kidney damage, mineralization disorders, and spontaneous fractures.

2. Calcitonin

Calcitonin is a type of calcium regulation hormone, which inhibits the biological activity of osteoclasts and reduces the number of osteoclasts. Another prominent feature of calcitonin is to alleviate bone pain, which is effective for bone pain caused

by osteoporosis and fractures. Currently, there are two kinds of calcitonin formulations for clinical application: elcitonin and salcatonin. Generally speaking, calcitonin is safe in clinic; a minority of patients may have side effect such as facial flushing, nausea, or allergic reaction.

3. Hormone replacement therapy (HRT) and estrogen receptor modulator

Hormone replacement therapy (HRT) mainly refers to estrogen-progesterone therapy, which mainly applies to postmenopausal women with high risk of fracture, especially women with menopausal symptoms such as hot flashes and night sweats. Estrogen receptor modulators bind to estrogen receptors on bone cells, play a role similar as estrogen to inhibit bone absorption, increase bone density, and reduce the risk of vertebral fracture.

4. Denosumab

Denosumab is the inhibitor of receptor activator of NF-κB ligand (RANKL), which inhibits the combination of RANKL and its receptor RANK. Denosumab reduces osteoclast formation, function, and survival, thus reducing bone resorption, increasing bone mass, and improving the intensity of cortical bone and cancellous bone.

(b) Anabolic drugs

1. Parathyroid analogue

Parathyroid analogue is a representative drug that is currently used to promote bone formation. Intermittent use of small doses of parathyroid analogue can stimulate osteoblast activity, promote bone formation and increase bone mineral density, and reduce the risk of vertebral and non-vertebral fractures.

2. Strontium salt

Strontium is one of the essential microelements in the human body; it participates in many physiological functions and biochemical effects. Strontium ranelate is a synthetic strontium salt, which simultaneously acts on both osteoblasts and osteoclasts. Strontium ranelate reduces the risk of vertebral and non-vertebral fracture through both inhibiting bone absorption and promoting bone formation.

3. Vitamin K

Vitamin K2 can improve the level of osteocalcin in blood; it is effective for both senile and postmenopausal osteoporosis. The common side effect of vitamin K2 is gastrointestinal reaction, and some patients may have pruritus and edema.

(c) Basic supplement

1. Calcium

Calcium is a basic supplement for bone health. Moderate calcium can reduce bone loss and improve bone mineralization. Calcium supplements have been shown to be beneficial for postmenopausal and osteoporosis. The 2013 dietary nutrient reference intake of Chinese residents recommended that the daily intake of calcium for adults was 800 mg, and the recommended daily intake of calcium for people aged 50 and above was 1000–1200 mg.

2. Vitamin D

Whether vitamin D supplementation reduces the incidence of fractures depends on the level of 25-hydroxyvitamin D in the body. Studies have shown that vitamin D supplementation can significantly reduce the occurrence of fractures when vitamin D is deficient. However, when vitamin D is sufficient, the function of vitamin D decreases. The recommended dose of vitamin D daily for adults is 200 U/day; for elderly and the lack of sunlight crowd, the recommended dose is 400–800 U/day. For the treatment of osteoporosis, the dose increases to 800–1200 U/day.

However, these drugs often have side effects, and patients cannot be completely cured under clinical treatment. The only anabolic drug for osteoporosis approved by the American PDA currently is tripeptides. The ultimate goal of developing anti-osteoporosis drugs is to rebuild the dynamic balance of osteoblast and osteoclast (Kim et al. 2012). Therefore, the development of drugs that are capable of both anti-bone resorption and anabolic functions is a trend, as well as a big challenge.

13.9 New Idea on Drug Development for Osteoporosis

Nacre forms the inner shell layer of some molluscan; it is a biologically produced organic-inorganic composite. Nacre consists of 95–99% aragonite and 1–5% organic matrix that contains proteins (Jackson et al. 1988), polysaccharide, pigment, and so on. Nacre is similar to the bone in both microstructure and function (Zhiyong et al. 2003), and it is notable for its biocompatibility among many other characteristics (Xu et al. 2001; Zhang et al. 2016); it is a widely used as a traditional Chinese mineral medicine.

It has been demonstrated that nacre has osteogenic activity after implantation into broken bones of many mammalian species such as rabbits (Lamghari et al. 2001a), rats (Liao et al. 2000), sheep (Atlan et al. 1999; Berland et al. 2005; Lamghari et al. 1999, 2001b), and humans (Atlan et al. 1997; Westbroek and Marin 1998) without causing increased inflammation. A series of osteogenic factors were found in nacre which can promote osteogenesis (Kim et al. 2012; Lopez et al. 1992;

Mouries et al. 2002; Rousseau et al. 2003, 2008). Water-soluble nacreous factors (WSNF), also called water-soluble matrix (WSM), extracted from nacre remarkably increases the level of bone-specific alkaline phosphatase (ALP) in mesenchymal stem cells (Lamghari et al. 1999), promotes extracellular matrix mineralization of pre-osteoblast cells (Chaturvedi et al. 2013; Rousseau et al. 2003, 2008), and decreases bone resorption by inhibiting osteoclast activity (Duplat et al. 2007; Kim et al. 2012).

Nacre growth is a biomineralization process which is mediated by some self-assembled organic materials (Almqvist et al. 1999; Stucky 1999); the organic materials formed a structural scaffold spontaneously around each single tablet of crystal nucleus and contributed to inorganic materials stacking (Aksay et al. 1996; Falini et al. 2011; John Spencer 2012). Proteins that function during this self-assembling process have similar sequence features, including interactive motifs and intrinsically disordered sequences (John Spencer 2012).

PFMG were a family of proteins that have been well studied about their influence on bone remodeling. Of which, PFMG1 was demonstrated not only to promote osteoblast proliferation, differentiation, and matrix mineralization but to prevent trabecular bone loss caused by ovariectomy (Li et al. 2018). PFMG1 proteins also share the similar structure with self-assembling proteins. PFMG1 contains two EF-hand calcium-binding domains near the C-terminus (Liu et al. 2007). The N-terminus of PFMG1 contains two amyloid-like cross-β strand supersecondary motifs; it is disordered and is capable of enhancing the aggregation tendency of the protein and facilitates matrix assembly processes (John Spencer 2012). Studies showed that PFMG1 affects the nucleation and aggregation of calcium crystals (Li et al. 2017; Liu et al. 2007); it is very likely that PFMG1 promotes matrix mineralization by accelerating calcium deposition and by promoting osteoblast differentiation and enhancing the secretion of bone matrix proteins (Li et al. 2018).

The study of nacre and nacre formation provides a powerful support that osteogenic factors or proteins like PFMG1 from nacre are suitable for the development of potential anti-osteoporosis drugs theoretically; considering their biocompatibility, it may also combine with bone substitution materials.

13.10 Conclusion

In conclusion, hormonal changes of ovaries highly correlated to chronological aging. The increased gonadotrophin levels and decreased estrogen were highly related to bone resorption, BMI, and lumbar bone mass. These results addressed that factors in addition to estrogen deficiency may probably have a role in bone metabolism in premenopausal women. We thought that studies about estrogen and bone metabolism would provide new insights into and would increase our understanding of osteoporosis in clinical practice.

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Biomarkers of Aging

14

Xiaojuan Bai

Abstract

Biomarkers of aging are a biological parameter that can predict the functional status of an individual in the absence of disease and can be used to better predict morbidity and mortality, compared to using the chronological age alone. Most of aging biomarkers were gene, molecules, and protein, which were found in basic scientific researches, such as telomeres, proteomics, cytokines, etc. However, it is almost impossible for single biomarkers to fully reveal the mechanism of aging. Because of the complexity of aging process, the biomarkers of aging may need to be composed of multiple genes, proteins, and metabolites. The biological age is based on the setting of biological markers, which is a parameter for evaluating the functional status of the individual. Aging is not only dependent on the process of time. The chronological age is only the evaluation indicators of time scale in the aging process. Therefore, biological age can be more representative of the true degree of aging than chronological age, which provides a quantitative standard for individualized aging. According to the factor score, we established biological age score (BAS) = 0.248 (CA) + 0.195 (IMT) - 0.196 (EDV) - 0.167 (E/A) - 0.166 (MVEL) + 0.188 (PP) + 0.182(FIB) + 0.193 (CYSC) through 7 aging biomarkers selected from 108 variables. The study found the rate of aging was gradually increased before the age of 75 years old and afterward entered a stable plateau. In the future, the new approach may be needed to investigate the mechanisms and evaluation of aging.

Keywords

Biomarkers of aging · Chronological age · Biological age · BAS

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14.1 Introduction

With an increase in average life expectancy and a decline in fertility, currently, the proportion and the absolute number of aging population are dramatically increasing in the world. In 2012, Japan was the only country where the people aged 60 and over accounted for more than 30% of the total population. However, according to the forecast by 2050, there will be many countries with the same proportion of older people as Japan of 2012; it includes not only many countries in Europe and North America but also Chile, China, Iran, South Korea, Russia, Thailand, and Vietnam by the middle of this century. At the same time, the pace of population aging is also significantly accelerated than ever. France has 150 years to adapt to the increase in the proportion of the elderly people with aged 60 years and over from 10% to 20%, while Brazil, China, and India have only 20 years to adapt to these changes. China is one of the fastest aging countries in the world. By 2013, the elderly population exceeded 200 million (accounted for 14.8% of the total population). By 2050, the elderly population will reach 437 million (Peng 2011), and the surging "white hair wave" is characterized by "geting old before geting rich" and resulting high prevalence of chronic disease. The population aging has become an important policy and economic issue.

From a biological point of view, aging was related to the accumulation of multiple injuries at the molecular and cellular level. Over time, these injuries gradually decreased the physiological reserves of the body, increased the risk of diseases, and reduced intrinsic capacity, eventually leading to death. Actually, aging is a complex process of biological system degeneration characterized by an irreversible accumulation and an increased susceptibility to disease in the body, eventually leading to the end of life (Zhang et al. 2013; De la Fuente 2008). Aging is not a disease, but it can change the threshold, severity, and prognosis of aging-related diseases by regulating the pathophysiological process of the disease. The age-related changes have the characteristics of nonlinear and heterogeneity, which show not only a certain degree of correlation with the individual aging. For example, 80-year-old individuals may differ biologically, while some enjoy good physical and cognitive function, others may suffer illness and require significant support to meet their basic needs. Therefore, the study of biomarkers and individualized evaluation of aging have important significance for the prevention and control of aging-related diseases and have become one of the hot spots in the field of aging.

14.2 The Concepts, Characteristics, and Trends of Aging Markers

In 1988, Sprott et al. firstly proposed the concept of biomarkers of aging, which is a biological parameter that can predict the functional status of an individual in the absence of disease and can better predict survival, compared to the chronological age (CA) (Sprott 1988). The American Federation for Aging Research has proposed the criteria for biomarker of aging as follows (Johnson 2006): (1) It must predict the

rate of aging. In other words, it would tell exactly where a person is in their total life span. It must be a better predictor of life span than chronological age. (2) It must monitor a basic process that underlies the aging process, without the effects of disease. (3) It must be able to be tested repeatedly without harming the person, for example, a blood test or an imaging technique. (4) It must be something that works in humans and in laboratory animals, such as mice. This is so that it can be tested in lab animals before being validated in humans. Because of the complexity of aging process, the biomarkers of aging may consist of multiple genes, proteins, and metabolites. The advantages of the combinational markers are not limited to the specific level of the organism. Compared with a single biomarker, the sensitivity and specificity of the combinational markers are significantly increased and are more likely to distinguish between subgroups, for example, the aging population with cardiovascular incidence and immune system decline has application value for aging diagnosis (Olovnikov 1973).

Since the early 1990s, a series of longitudinal studies on aging have been carried out in developed countries, representative studies included Baltimore Longitudinal Study of Aging (BLSA) and Health, Aging, and Body Composition (Health ABC) study in United States (Ferrucci 2008; Njajou et al. 2009), the Victoria Longitudinal Study in Canada (MacDonald et al. 2004), the Italian Longitudinal Study on Aging in Italy (Inzitari et al. 2006), and the 7-year longitudinal study in Japan (Nakamura and Miyao 2008). It can be seen from the research trends: (1) The hot spots of research have shifted to healthy aging and emphasize the individualized evaluation of aging; (2) it is an important topic to screen reliable and easy-to-test biomarkers of aging; (3) the use of systematic biological methods to establish the whole aging nonlinear network structure model is the future research direction; and (4) the genetic background, lifestyle, and dietary are important factors for affecting individual aging.

Facing the increasingly severe challenge of population aging, China started the National Program on Key Basic Researck Project (973 Program) since 2000, with academician Chen Xiangmei assumed as chief scientist. One of the main research directions of this project was the exploration of new biomarkers of aging. Actually, the biomarkers of aging can be divided into two categories according to their role and target: one is the molecular and cellular level marker and the other is the phenotype and functional evaluation of aging; there is significant difference between these both methods. Our team undertook the study of biomarkers of aging belonged to the latter in the 973 Program.

14.3 The Clinical Application of Aging Biomarkers: Aging Evaluation and Early Warning of Age-Related Diseases

14.3.1 A Combination of Biomarkers: The Significance of Biological Age

Biological aging is a highly individualized process; due to the effects of genetic, environmental, and disease factors, the biological age (BA) is very different from any given CA among individuals; and eventually it can be expected to be consistent

with individual longevity and the rate and/or the magnitude of the aging process. Some people have very good physical and cognitive function at the age of 85 years, but some people have a wide range of cognitive or physiological dysfunction at the age of 65 years, so the CA is only a rough evaluation index in the aging process. From the perspective of medicine and prevention, the researchers are mainly concerned with the changes of individual aging; the purpose is to screen high-risk individual and make timely intervention. Therefore, some researchers put forward using BA instead of CA for evaluation of individual aging (Anstey et al. 1996). Our team's study also confirmed that biological age (BA) calculated by using BA equation constructed on the basis of the biomarkers of aging was superior to CA for evaluation of individual aging (Bai et al. 2010). BA is a parameter for evaluating the functional status of the individual on the basis of the functional status of their peers relative to the CA (Borkan and Norris 1980). The concept of BA is based on the setting of biomarkers that the passage of time is only indirectly related to aging, which is not dependent on the process of time. Therefore, BA can be more representative of the true degree of aging than CA, which provides a quantitative standard for individualized aging.

BA is superior to CA in evaluating biological aging (Bai et al. 2010; Nakamura and Tanaka 1998). However, the aging is a relative concept. There is no good method to determine the normal aging range of healthy people in the literature. Currently, the healthy aging is a hot topic of aging research internationally; the goal is to explore and prolong the energetic disease-free age stage and to shorten the age stage of diseases and dysfunctions in life span. The long and short of simple measurement life span did not provide enough information in assessing the effectiveness of the intervention on aging and age-related diseases. The role of BA is similar to some evaluation criteria used in other complex systems (such as Apgar score), with the aid of which the researchers can assess, summarize, and simplify some information of the system so as to make it more useful (Miller 2002). A link has been made between mortality (the indicators of population level) and the aging-related changes of the different physiologic indicators (an individual-level phenomenon). BA has now been proposed as a predictor of the individual general health status and a healthy life expectancy (the survival time without dysfunction); it helps to identify individuals with the risk of age-related dysfunction and to predict late-life dysfunction and death independent of CA (Borkan and Norris 1980; Mitnitski et al. 2002; Uttley and Crawford 1994).

14.3.2 A Comparison of Biological Age Methods for Evaluating Aging

The statistical methods play an important role in the study of biological age evaluation, which determines the validity, specificity, and limitations of the BA model. The multiple linear regression (MLR) method (Dubina et al. 1983), the principal component analysis (PCA) method (Bai et al. 2010; Zhang et al. 2014a, b), the Hochschild method (Hochschild 1989), and the Klemera and Doubal method

(KDM) (Klemera and Doubal 2006) were applied to construct BA models, but their advantages and disadvantages are different. No matter what method, the core concept of assessing BA is factor analysis. The purpose is to explain the basic covariance structure through a series of observation variables to find out the potential dominant factors. In general, the greater the factor loading of a variable on a factor, the more important it is to interpret this factor. In other words, factor analysis is the screening and selection of biomarkers of aging.

A major problem of the multiple linear regression is the existence of redundant predictors. Because of complex organisms and physiological processes are interdependent, there is redundancy in complementary systems of maintain function (Mitnitski et al. 2002; Nakamura et al. 1989). Another problem of the multiple linear regression is that it overestimates the BA of the young group and underestimates the BA of the elderly group. Dubina et al. (1983) found that the error was due to the mathematical properties of linear regression. The purpose of multiple regression analysis is to obtain prediction equation of dependent variable as accurate as possible. Because the indicators of constructing BA were linear correlation with CA, it is often difficult to determine whether the CA is the biomarker of aging or the main selection criteria. Moreover, a number of studies were applied using multiple regression equation to predict BA, it was made CA as dependent variable, and as a result, the prediction of regression equation is CA of individuals, rather than BA.

The principal component analysis method can be defined as the linear combination of optimal weights of observational variables in order to integrate a large number of observational variables into a couple of comprehensive variables. Meanwhile, the variance explained by the original variables is maximized to obtain a simplified and generalized comprehensive indicator. Nakamura et al. (1989) constructed the BA equation by using PCA method in 7-year longitudinal study in Japan. In China, our team firstly proposed the application of PCA method to study biomarkers of aging and to construct the BA model. Based on population data from a single center or multiple cities in China, we have constructed and repetitiously validated the biological age score (BAS) (Bai et al. 2010; Zhang et al. 2014a, b). For nearly 10 years, we added new indicators and sample size each time to study the compatibility of BA models, but by comparing our different BA models, some biomarkers, such as kidney function indicate (CYSC) and the indicators of vascular structure and function (carotid intima-media thickness), have been used (Bai et al. 2010; Zhang et al. 2014b, 2017). This not only suggests the stability of PCA in selecting the biomarkers of aging but also can be used as aging indicators for cardiovascular structure and kidney. Those biomarkers play an important role in the construction of BA. Famous scholars have suggested that the essence of senescence is vascular aging (Lakatta 2000).

Researchers usually reduce the number of age-related variables to simplify the analysis through MLR and PCA methods. The essence of selecting the biomarkers of aging is usually associated with the CA in these two methods. Over the years, our team has carried out studies on kidney aging (Wang et al. 2016b; Jiang et al. 2012), bone aging (Han et al. 2017; Liang et al. 2014), and gastric function aging (Shan et al. 2017). Although the results suggested that the biomarkers of aging were

significantly associated with age in these organs, many indicators of organ aging did not use linear regression equation in the process of constructing the overall biological age score (BAS). Therefore, it is very important to evaluate the methodology of biological age. Some scholars point out that the correlation with CA is not a reasonable standard for selecting, validating, or weighting the biomarkers of aging (Ingram 1988). In addition, many biomarkers were moderately correlated with CA, but in fact they were not associated with aging (Barak and Schiffman 1981). Hochschild et al. (Hochschild 1989) considered it inappropriate and unreasonable to define CA as the selection criterion. However, completely avoiding the use of CA does not improve the results and lead to a complete loss of important information.

Hochschild puts forward a new method to solve these problems, based on the impact of the life expectancy to select biomarkers of aging, because this method is a nonstandard and relatively complex method, which is rarely used in the evaluation of BA, but it provided a novel idea on BA for us. KDM is a graphical approach proposed by Klemera and Doubal (2006) in 2006. It is described as the optimal method to evaluate BA, even suitable for young people (Belsky et al. 2015). Subsequent studies have confirmed that the BA constructed by KDM is more reliable in predicting mortality (Jee and Park 2017; Levine and Crimmins 2014; Levine 2013). However, the application of KDM and its complexity are only applicable to the research teams of computer and statistical support. In addition, whether or not to include CA as a biomarker of aging remains controversial. Mitnitski et al. (2017) reported that the evaluation of BA without CA is most appropriate. At present, it is generally considered the best method for BA evaluation, but the conclusions are not accurate, because mortality and disease only reflect certain aspects of the effectiveness in BA evaluation. Biomarkers of predicting mortality and longevity are not necessarily reciprocal, and they may be completely different biomarkers, such as telomeres (Blackburn et al. 2015). The longevity and life expectancy are determined by many factors, including the environment, lifestyle, genetic factors (Bulpitt 1995).

14.3.3 The Construction of Biological Age Formula (Bai et al. 2010)

Aging is a highly individualized process, and the most important feature is the individual difference of physiological function degeneration (MacDonald et al. 2004). CA is only the evaluation indicators of time level in the aging process. In fact, BA is more representative of the true extent of the aging process than CA. Under the direct guidance of academician Chen Xiangmei, our team screened 2876 subjects 30–98 years of age from three Chinese cities (including Shenyang, Beijing, and Dalian), and finally 852 healthy subjects were selected. They were divided into four groups according to their age as follows: young group (<45 years old), middle-aged group (45–59 years old), old group (60–74 years old), and very old group (75 years old). The intima-media thickness (IMT), end diastolic velocity (EDV), arterial pulse pressure (PP), ratio of peak velocity of early filling to atrial filling (E/A), mitral valve annulus lateral wall of peak velocity of early filling (MVEL), cystatin C

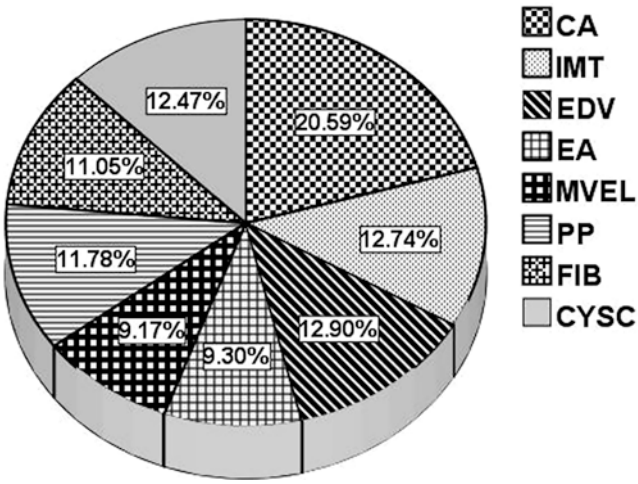

Fig. 14.1 Contribution of each variable to the variance of BAS according to its vector of factor loadings of the single factor. (Modified with permission from Bai et al. 2010. Copyright 2009 S. Karger AG, Basel. All rights reserved)

(CYSC), and fibrinogen (FIB) were selected as biomarkers of aging from a total of 108 variables. Factor analysis showed that there was only one factor with eigenvalue greater than 1; it was suggested that these seven biomarkers collectively reflect the basic process of aging. According to the factor score, we established biological age score of the optimization BAS = 0.248 (CA) + 0.195 (IMT) - 0.196 (EDV) - 0.167 (E / A) - 0.166 (MVEL) + 0.188 (PP) + 0.182(FIB) + 0.193 (CYSC). The correlation coefficient between the BAS calculated by this formula and the CA was 0.893, $p < 0.001$. It can be seen from the composition of the formula that there is a close correlation between individualized aging and individual vascular structure (IMT), pulse pressure (PP) and function (EDV), cardiac diastolic function (E/A, MVEL), inflammatory status (FIB), and renal function (CYSC). In the evaluation of aging, cardiovascular markers were possessed of an important position; the indicators of cardiac diastolic function is more important than the systolic function, because cardiac systolic function changes with age are small. Arterial structures and functions changed significantly with age. Inflammatory markers also played an important role in BAS. Figure 14.1 showed the contribution of each variable to the variance of BAS according to its vector of factor loadings of the single factor. Figure 14.2 showed the correlation of chronological age and biological age scores.

14.3.4 Evaluation of the Degree and Rate of Aging

One of the important clinical applications of aging biomarker research is the aging degree and rate in individual evaluation, to provide target populations and targets for the early intervention of clinical aging and geriatric disease and to achieve "center of gravity forward" in the prevention and treatment of age-related diseases. Our

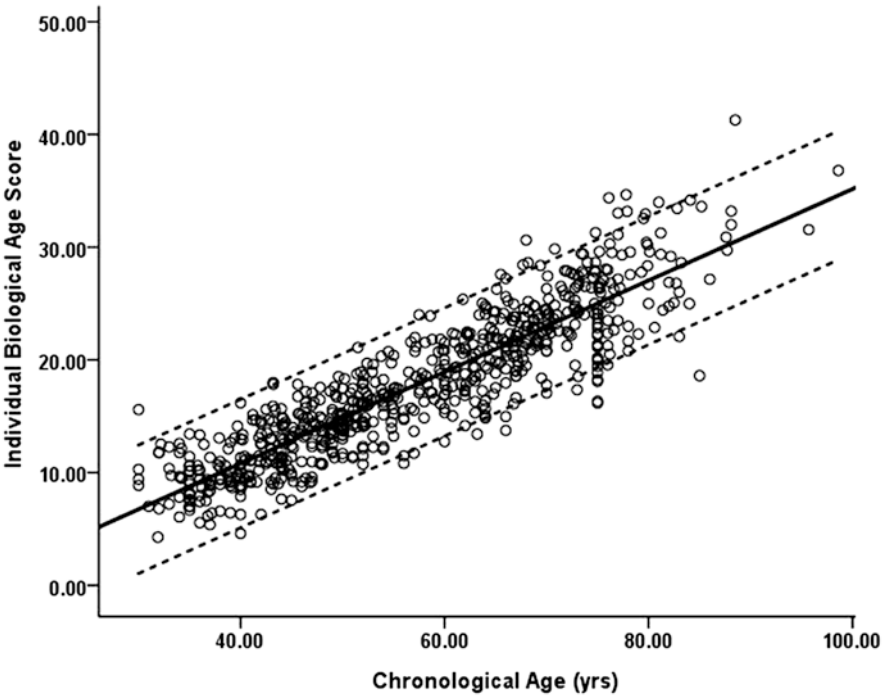

Fig. 14.2 Relation between BAS and CA. BAS is predicted from the sum of the follow variables: BAS = 0.248 (CA) + 0.195 (IMT) – 0.196 (EDV) – 0.167 (E / A) – 0.166 (MVEL) + 0.188 (PP) + 0.182(FIB) + 0.193 (CYSC). Solid and dotted lines indicate the correlation of BAS with CA and its 95% confidence interval, respectively ($r = 0.893$, $p < 0.001$). (Modified with permission from Bai et al. 2010. Copyright 2009 S. Karger AG, Basel. All rights reserved)

team screened 7 aging biomarkers and constructed the BA equation, by which we evaluated the BA of 852 healthy individuals. Linear regression was performed using BAS as the dependent variable and CA as independent variables; based on the confidence interval of regression line ±1 standard deviations, the 852 subjects were divided into delayed aging group, normal aging group, and premature aging group. Meanwhile, we observed the difference of seven biomarkers in different degree age groups; the result showed that there was no significant difference in the CA of the same age group after grouped according to the degree of aging, but there were significant differences in the BAS among groups. Seven biomarkers have significant difference between different CA groups and different aging degree groups. The biomarkers have significant difference among the four age groups. It fully demonstrated that BAS played an important role in the evaluation of aging. In the same CA population, its biological aging degree is inconsistent. Significant outcome will be obtained if the necessary intervention was carried out in the premature aging population. Our results indicate that the aging rate is slowest in the age group of <45 years, rapidly accelerated at 45–59 years, and reached the peak at age group of 60–74 years, and then the aging rate remains at stable plateau (Fig. 14.3).

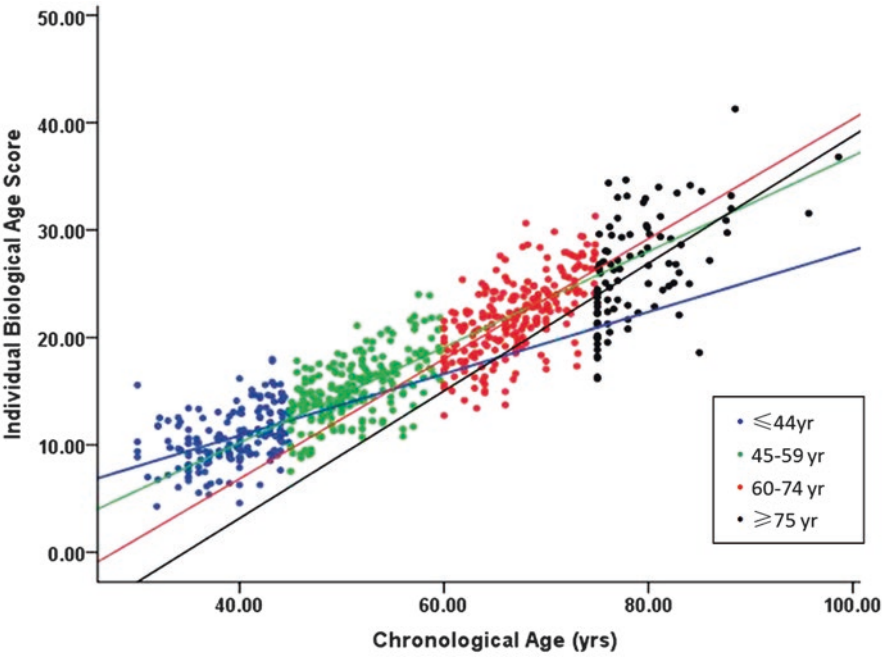

Fig. 14.3 Relationship between BAS and CA with stratification of different age groups. The slopes of the regression line of BAS to CA for the young ($\beta = 0.287$, $p < 0.001$), middle-aged ($\beta = 0.445$, $p < 0.001$), old ($\beta = 0.558$, $p < 0.001$), and very old ($\beta = 0.593$, $p < 0.001$) groups are significantly different. Post hoc analysis indicates a significant difference in the slopes between the young and middle-aged groups, between the middle-aged and old groups, between the middle-aged and very old groups, but not between the old and very old groups. (Modified with permission from Bai et al. 2010. Copyright 2009 S. Karger AG, Basel. All rights reserved)

The rate of aging is closely related to human life expectancy and aging-related diseases. According to Holliday, the faster the rate of aging is, the higher the likelihood of the occurrence and death of aging-related diseases. Delaying the aging rate of individuals ultimately benefits the achievement of healthy aging. Understanding the difference of aging rate and the regular change with CA is of great importance in the disease prevention and healthy aging.

14.4 Basic Research on Aging Biomarkers: Monitoring the Process and Mechanism of Aging

14.4.1 Telomere Length and Aging

Cell senescence is a consequence, which the changes of cellular structure and function accumulate to a certain extent. The replication phase of cells is considered to be determined by two mechanisms of aging, one of which is the progressive shortening

of telomeres. In 1973, Olovnikov et al. (Olovnikov 1973) for the first time put forward the theory that the loss of telomere was associated with senescence, suggesting that telomere loss caused by terminal replication might regulate the life span of cell. Bodnar et al. (1998) prolonged the life cycle of normal human cells by activating telomerase activity to extend telomeres and finally demonstrated the relationship between telomere and aging. The 2009 Nobel Prize in Physiology and Medicine was awarded to three American scientists who made outstanding contributions in the field of telomere and telomerase. So far, the study of telomere and telomerase has become one of the hot topics for research on the mechanism of aging in the world. A large number of studies also confirmed that telomere length gradually shortened with aging. In addition, the extended guanine-rich sequences by telomerase reverse transcriptase on telomere DNA were able to form G-quadruplexes in vivo and in vitro, the Tan Professor team in China analyzed the real conformation of telomere end G-quadruplexes structure, and it was found that a stable G-quadruplex was formed only in a molecularly crowded environment created by the physiological concentration of polyethylene glycol (PEG) (Zheng et al. 2010). In addition, the team applied the real-time fluorescent assay and BLM helicase to investigate the intramolecular unwinding of the G-quadruplex; the study found that the intramolecular G-quadruplex unfolding efficiency was related to the structural stability, as well as much lower than the corresponding duplex substrates, and revealed that the G-quadruplex regulates DNA and RNA in a stable and dependent manner (Liu et al. 2010). As a result, if the telomere is shortened, the cells will age; on the contrary, telomere length can be maintained by telomerase activity increase and can delay cell senescence. So it is certain that telomere length change is one of the biomarkers of human aging.

14.4.2 DNA-Related Biomarkers of Aging

Another mechanism of cell senescence is the accumulation of DNA damage; the constant attack of the exogenous and endogenous factors [including reactive oxygen species (ROS)] can damage NDA and disturb epigenetic regulation. The DNA repair system and DNA methyltransferase are also involved in the maintenance of sustainable molecular damage levels. DNA damage was considered to be the result of aging, due to the damage of antioxidant defense mechanisms and DNA repair capacity (Hazane et al. 2006). The mitochondria of human cells produce more oxygen free radicals during aging process, which in turn damage the DNA, especially oxidative DNA damage caused by reactive oxygen species that can form 8-hydroxydeoxyganosine (8-OH-dG) complex with bases; the mass production of the latter causes the base mismatch in the process of DNA replication, resulting in mutations (Hamilton et al. 2001). Since Linnane et al. (1989) proposed the hypothesis of mitochondrial senescence in 1989, more and more people focused on the study of the relationship between mitochondrial DNA (mtDNA) mutations and senescence. Because mtDNA mutations are particularly vulnerable targets, mitochondrial DNA damage and mutations are also considered as the main mechanisms of driving the

senescence process (Altilia et al. 2012; Wong et al. 2009). Wiesner et al. (2006) suggested that mtDNA mutation exceeded mtDNA repair ability as an important molecular mechanism of human aging, which may be dependent on senescence caused by mtDNA deletion. In age-related degenerative diseases, mitochondrial dysfunction occurred in the early stages of disease and contributed to the pathogenesis (Lin and Beal 2006; Tanaka 2002). In addition, the mtDNA mutation sites may be related to longevity. Niemi et al. (2005) found that the mtDNA5178 site purine/cytosine polymorphism was correlated with longevity/aging in the longevity of the elderly in Japan; meanwhile, the haplotypes J2, D5, and M7b were associated with longevity, and the mtDNA contains 150T site mutation, indicating that the polymorphism of 150T site may be related to longevity.

14.4.3 Gene-Related Biomarkers of Aging

Human silent information regulator type 1 (SIRT1) is an important determinant of longevity and plays an important role in the regulation of life span of different species (Guarente and Kenyon 2000; Nemoto et al. 2004). The peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-activated transcription factor belonging to the steroid receptor superfamily and plays a key role in inducing cell differentiation and inhibiting cell growth by promoting cell cycle stagnation (Chang and Szabo 2000); And SIRT1 is the molecular link between cellular senescence and overall aging, it directly interacts with PPARγ and inhibits the activity of SIRT1 at the transcriptional level and forms a negative feedback and self-regulatory loop, and it was shown that the acetylation and deacetylation regulation of PPARγ were regulated by the histone acetyltransferases P300 and SIRT1 in cell aging (Han et al. 2010), providing an important target for the intervention of aging. Zhang et al. (2010) reported that the rs4746720 site of SIRT1 gene is associated with aging; it was suggested that SIRT1 gene polymorphism may increase the impact of multifactorial genetic contributions on aging. The expression of p^{16} anti-oncogene was significantly increased when cell senescence. Transferring the p^{16}cDNA recombinant vector into normal human fibroblasts could slow down cell growth, aggravate non-enzymatic glycosylation, increase the activity of β-galactosidase, and shorten telomeres (Tsutsui et al. 2002). Borzillo et al. (1992) inserted the B-cell lymphoma-2 (Bcl-2) gene into the expression vector and found that the survival time of Bcl-2 transgenic cell lines were prolonged significantly in various adverse conditions, which suggested that Bcl-2 was involved in cellular senescence. The overexpression of Bcl-2 prolonged the lifespan of B cells and various hematopoietic cells after removing growth factors (O'Reilly et al. 1997). Lope et al. (Lopez-Diazguerrero et al. 2006) found that overexpression of Bcl-2 can protect cells from proliferation decline, improve the viability of cells, and delay cell senescence. Because the Bcl-2 has the function of inhibiting apoptosis and prolonging cell survival, it is called longevity gene, but the cell survival depends on the expression of apoptosis gene and apoptosis-suppressing gene.

14.4.4 Cytokine-Related Biomarkers of Aging

In the research of immune senescence, some cell inflammatory factors play a decisive role in the process of aging. The activation of local inflammation and the change of gene expression profile of different inflammatory cytokines may be an important factor in the senescence of the body. At present, the relationship between cytokines and aging is also widely studied. The age-related pro-inflammatory factors included interleukin-6 (IL-6), tumor necrosis factor-a (TNF-a), and C-reactive protein (CRP) (Franceschi et al. 2000). Many previous studies have shown that serum IL-6 concentration increases with age (Hager et al. 1994; McKane et al. 1994). A cross-sectional study of 396 subjects (more than 50 years old) in Wisconsin found that serum IL-6 levels were associated with age-related cataracts (Klein et al. 2006). The higher plasma concentrations of IL-6 and TNF-α are associated with decreased grip strength and gait speed; it is suggested that the immune and functional status are interrelated in the elderly (Visser et al. 2002). Schaap et al. (2006) found that high IL-6 (>5 ng/L) and high C-reactive protein (CRP, >6.1 mg/L) were associated with a 2 to 3-fold greater risk of losing greater than 40% of muscle strength, which indicated that higher levels of IL-6 and CRP increased the risk of muscle strength loss. A meta-analysis found that high CRP concentration was consistently associated with the risk of age-related diseases including coronary heart disease, ischemic stroke, a variety of cancers, and lung disease (Kaptoge et al. 2010). It is obvious that these inflammatory factors have many nonclassical functions; it is far beyond the traditional significance of inflammatory function in the process of metabolic regulation. Therefore, inflammatory factors predict the decline of chronic inflammatory state and immune function of the body, which may be good biomarkers to reflect aging susceptibility of the elderly.

14.4.5 Molecular Protein-Related Biomarkers of Aging

Because of the complexity of the body's aging process, a large number of proteins are also involved in the aging processes of cells and organs. The team of academician Tong of Peking University autonomously cloned encoding cellular senescence-inhibited gene (CSIG) protein. This study showed that phosphatase gene (PTEN gene) was necessary to be involved in the process of the CSIG regulating p27Kip1 expression and cell cycle, and then the GSIG interacted with the 5-untranslated region (UTR) of PTEN gene mRNA and negatively regulates PTEN gene at the translational level, which can significantly delay the process of cell replicative senescence when overexpressed (Ma et al. 2008). In the study of organ aging, the team of academician Chen Xiangmei from the Chinese People's Liberation Army General Hospital reported that the mammalian target of rapamycin (mTOR) increased with age in the kidney and may promote cell senescence by p21WAF1/CIP1/SD11 regulating cell cycle. Growth differentiation factor 11 (GDF11), a secreted protein of the transforming growth factor β(TGF-β) superfamily member, is also called bone morphogenetic protein 11 (BMP11). In general, GDF11 was

expressed in many tissues and organs of the human body as age-related circulating factor (Uhlen et al. 2015). Many recent studies have shown that serum GDF11 levels are closely related to the development of some age-related diseases. For example, Loffredo et al. (2013) reported that GDF11 levels reversed age-related cardiac hypertrophy and declined with age in mice. Another study found that GDF11 improves cerebral blood flow, enhances neuronal function and activity (Katsimpardi et al. 2014), and offers new insights into the treatment of age-related neurodegenerative diseases. Moreover, Sinha et al. reported that the GDF11 levels generally decreased with age. After supplemented with GDF11, the structure and function of skeletal muscle of mice were improved and strength and endurance of skeletal muscle were enhanced. These results showed that GDF11 systematically regulated muscle aging (Sinha et al. 2014) and maybe a therapeutic target for reversing aging-related skeletal muscle dysfunction. In addition, the fibroblast growth factor-23 (FGF23) (Wang et al. 2017; Dalal et al. 2011), the cathepsin B (CTSB) (Wang et al. 2016a), the insulin growth factor-1 (Li and Ren 2007; Bluher et al. 2003), cystatin C (CYSC), and fibrinogen (FIB) (Drenos et al. 2007) were also closely related to aging.

14.5 Future Research Direction

14.5.1 Prospective Population Research and the Development of Simple-Easy Aging Evaluation Formula

Data showed that nearly two-third of lifetime medical expenditures was incurred during the senior years. Elderly patients account for 60% of emergency visits, 49% of hospital stays, and 85% of long-term care beds, the consumption of health resources is 1.9 times the average health-care resources of the entire population, and substantial resources are made using the later stages of disease, resulting in a rapid increase of disease burden of an elderly patient. A prospective cohort study was conducted to optimize aging biomarker system of the combination that could predict the risk of age-related disease morbidity and mortality. Especially a simplified aging assessment method for the application of primary medical institutions was developed. There are plans and steps to systematically carry out individualized evaluation and stratification of aging in different levels of hospitals and community hospitals. The purpose is to screen out high-risk population of aging and to conduct targeted interventions. It can help to achieve the "center of gravity forward" of age-related disease prevention and targeted management; the efficient use of limited health resources will be the focus of future research.

14.5.2 Respect the Characteristics of Nonlinear Network of Aging: Neural Network Combined with Aging Research

Aging-related changes have the characteristics of nonlinear and heterogeneity, and the overall aging is not a simple combination of organ and tissue aging but showing

the complex network process through the connections and integration of molecules, cells, tissues, and organs, and it involves complex social factors and lifestyles and behaviors of human being, which are systematic and complex (Kriete et al. 2006; Kirkwood 2008). Therefore, to solve this complicated process of biological aging, we should reply on artificial intelligence with a systematic approach to study the biological aging. The organism is seen as a whole that consists of interacting and interrelated elements; meanwhile, only when we study the diversity, function, and interaction networks of these elements, it is possible to better understand the ways and mechanisms of the systematic aging. Artificial neural networks are a mathematical method of mimicking neurons in the biological nervous system. Because of the ability of parallel processing, self-organization, self-learning, associative memory, and fault tolerance, artificial neyral network can be served as an expert system with good performance in classification diagnosis, intelligent control based on classification, and optimization solution, which is more superior for processing the information of aging biomarkers of human being. Neural networks have had some applications in medical diagnostics, such as high molecular sequence analysis in biomedicine, image analysis, and auxiliary diagnosis. At present, the application of neural network in the field of aging research is only just the beginning, but this has attracted more and more attention of biomedical researchers.

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Pharmaceutical Intervention of Aging

15

Minxian Qian and Baohua Liu

Abstract

The aging population represents a significant worldwide socioeconomic challenge. Aging is an inevitable and multifactorial biological process and primary risk factor for most age-related diseases, such as cardiovascular diseases, cancers, type 2 diabetes mellitus (T2DM), and neurodegenerative diseases. Pharmacological interventions targeting aging appear to be a more effective approach in preventing age-related disorders compared with the treatments targeted to specific disease. In this chapter, we focus on the latest findings on molecular compounds that mimic caloric restriction (CR), supplement nicotinamide adenine dinucleotide (NAD^+) levels, and eliminate senescent cells, including metformin, resveratrol, spermidine, rapamycin, NAD^+ boosters, as well as senolytics. All these interventions modulate the determinants and pathways responsible for aging/longevity, such as the kinase target of rapamycin (TOR), AMP-activated protein kinase (AMPK), sirtuins, and insulin-like growth factor (IGF-1) signaling (Fig. 15.1).

Keywords

Aging · Age-related diseases · Pharmaceutical intervention

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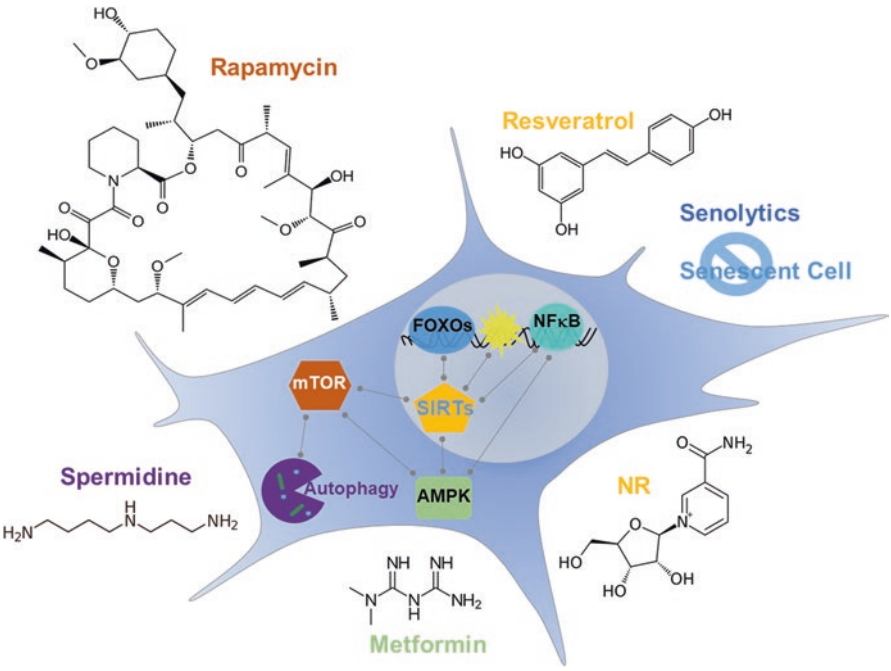


Fig. 15.1 Pharmaceutically targeted genes/pathways of aging/longevity

15.1 Introduction

Over the past 50 years, profited from improvements in public Medicare systems and living conditions, the life expectancy has remarkably prolonged. As a consequence, most modern nations are facing rapid population aging. It is conceivable that more than two billion of the global population will be over the age of 60 by 2050. Aging is inevitable and irreversible, accompanied with loss of viability but increase in vulnerability. It remains the predominant risk factor for the majority of life-threatening diseases, such as heart diseases, cancers, and Alzheimer's diseases. The proportion of older people suffering from age-related diseases will reach to almost double over the next two decades. In industrialized countries, age-related diseases are already on the top list of causes to death. Inevitably, their prevalence will continuously increase (Butler et al. 2008; Olshansky 2006). Therefore, developing approaches and therapies intervening aging and aging-related diseases is a great and urgent need (Butler et al. 2008).

The increasing common sense is that the incidence of multiple pathologies is strongly contributed to the progressive deterioration of physiological function with age, and targeting aging process is a highly efficient way for treating age-related diseases. Long-lived mutants, such as naked mole rat and centenarians, exhibit the late onset of many age-related diseases. Slowing down aging means not only to increase life span but to reduce the prevalence of comorbidities, resulting in the

extension of healthspan. Delaying the process of aging, even slightly, would produce profound socioeconomic and health benefits (Butler et al. 2008; Olshansky 2006). Life extension by a mere 7 years would decrease mortality by half at every age, mostly due to the late onset of age-related diseases.

During the past decades, various genetic interventions, pharmaceuticals, and lifestyle have been shown able to increase life span and/or healthspan in model organisms. More than 700 genes associated with aging and longevity have been identified (de Magalhaes et al. 2009). For notable instance, worms with *daf-2* mutations live more than twice as long as their wild-type siblings (Kenyon et al. 1993). Mutating or inhibiting S6 kinase has positive impacts on life span of yeast, worms, flies, and mice (Kaeberlein et al. 2005; Kapahi et al. 2004; Pan et al. 2007; Selman et al. 2009). It is now known that gene manipulations extend the life span and/or healthspan of invertebrates and mammals by altering the reproductive system, reducing caloric intake, modulating hormone levels, and altering insulin/IGF1 signaling pathways. However, these specific manipulations are unlikely to be directly applicable to humans, while they may shine the road for developing drugs against aging process and aging-related diseases.

Caloric restriction (CR), also called dietary restriction, is the most widely studied and conserved intervention that extends life span and improves healthspan in almost all organisms. Studies in nematodes and flies suggest that nutrient deprivation triggers life span extension and reduces mortality even when intervened late in life (Mair et al. 2003; Spindler 2005). In rodents, CR can extend life span by up to 50% and delay or even diminish the morbidity of most aging-related diseases, including immune diseases, neurological diseases, diabetes, cancer, as well as cardiovascular diseases. Notably, CR reduces body fat and inflammation in primates, in addition to delaying the onset of age-related diseases (Colman et al. 2009). Mechanistically, CR promotes longevity via activating sirtuins and AMPK, as well as inhibiting the insulin and TOR pathways, which both lead to stress resistance and autophagy activation (Fontana et al. 2010). It is noteworthy that cellular responses to CR are complex, much less in primates. Despite its health and life-extending outcomes, in humans, long-term CR may provide negative functional consequences and potential side effects, such as an impaired glucose tolerance, a reduction in fecundity and muscle mass, and slower wound healing (Dirks and Leeuwenburgh 2004, 2006; Fontana et al. 2010; Partridge et al. 2005). Clearly, the disadvantage of CR is problematic to encourage people generally establish such dietary regiment as a suitable strategy in our current society to keep health. Therefore, CR as a paradigm for developing CR mimetics or foods without its side effects is of immense scientific, social, and commercial interest (Ingram et al. 2006).

Screening or designing compounds, based on the molecular mechanisms underlying aging and interventions that promote longevity, is the most straightforward and widely explored approach to target aging and its related diseases. For instance, pharmacological activation of SIRT1 (resveratrol or SRT2104) and inhibition of mTOR (rapamycin) have been explored to prolong longevity and improve healthspan across species. Some drugs that exert as CR mimetics (CRM) strikingly produce promising results in current clinical trials. More recently, bioinformatics

analysis has enlarged the existing pool of CRMs (Calvert et al. 2016). Recently, the pro-longevity benefits of nicotinamide adenine dinucleotide (NAD^+) supplement, accompanying with improved organ function and disease resistance, are drawing great attention to design compounds that enhance NAD^+ levels, which decline with age. New preclinical compounds, called "senolytics" to selectively target senescent cells, which accumulated with age potentially causing fatal diseases, were first explored in 2015 and rapidly spring up in the following years. It is worth pointing out, by the investigators, a truth that the pharmacological interventions on aging should meet three highly selective criteria: significantly extending life span and/or healthspan, validated in at least three model organisms, and confirmed by at least three independent laboratories (de Cabo et al. 2014). Thus, we know only a few low-molecular-weight compounds are applicable to trigger life span extension via epigenetic alterations (histone acetylation/methylation), the insulin/IGF-1 pathway, TOR signaling pathway, mitochondrial function, proteostasis, autophagy, and stress resistance. Here, we summarize current knowledge on these promising approaches and critically evaluate their druggable applicability as antiaging interventions.

15.2 2-Deoxy-D-Glucose (2DOG)

2DOG is a chemical inhibitor of glycolysis, which extends life span of nematodes in a similar manner as CR (Schulz et al. 2007). By competing with glucose, 2DOG is uptaken by the cells but cannot be catabolized. Feeding or injection of 2DOG in rodents induces mitochondrial respiration, activates AMPK, and increases ROS production. Despite the beneficial effects of 2DOG on cancer (Kang and Hwang 2006), Alzheimer's disease (Yao et al. 2011), and Parkinson's disease at low doses, it has been shown that 2DOG treatment caused heart failure in rats due to its cardiotoxicity, and the life-extending effects on rodents are not conclusive yet. D-glucosamine (GlcN), a structure related to 2DOG, significantly prolongs life span in nematodes by inhibiting glycolysis (Weimer et al. 2014). When applying to a mouse model, Weimer et al. observed that GlcN-treated aged mice exhibited increased life span, without effects on body weight and diet intake (Weimer et al. 2014). Similar to 2DOG, GlcN can be effective against a wide range of cancer cells. Another recent study has provided evidence that GlcN activates autophagy (Carames et al. 2013), an essential mechanism of cellular homeostasis that will be discussed in the final section. Given the impressive safety profile and pro-longevity effects of GlcN, clinical studies on its effects on healthspan report will be undoubtedly ongoing.

15.3 Rapamycin

Rapamycin, also termed as sirolimus from the bacterium *Streptomyces hygroscopicus*, is an early example of a licensed drug proving to have a wider therapeutic range. The compound was generally considered on the basis of its known property as a specific inhibitor of mTOR (target of rapamycin). Rapamycin associates with

the intracellular protein FKBP12 and inhibits mTORC1 activity by blocking the interaction between mTOR and raptor. The replicative life span of yeast with *tor1* mutant is extended (Kaeberlein 2007). Inhibiting mTOR to slow mammalian aging process was first indicated by the National Institute on Aging's Interventions Testing Program (ITP). Mice lacking ribosomal S6 protein kinase 1 (S6K1), a downstream component of the TOR pathway, live longer and exhibit resistance to age-related pathologies (Selman et al. 2009). Administration of rapamycin in *Drosophila* robustly extends life span by the TORC1-dependent downstream processes, autophagy and protein translation (Bjedov et al. 2010). In mice treated with rapamycin from 6 months of age, the mean life span was significantly increased (10–18%) (Miller et al. 2011). Deficiency of Bmal1, a transcriptional factor and core component of the circadian clock, reduced life span of mice, which can be restored by rapamycin treatment by 50% (Khapre et al. 2014). Like dietary restriction, rapamycin-inhibiting TOR activity not only extends life span of yeast, worms (Stanfel et al. 2009), or mice but also delays the incidence of age-associated decline and disease in invertebrate and rodent models, including poor hematopoietic system, neurodegenerative disorders, cardiovascular dysfunction, obesity, and cancer (Chen et al. 2009; Harrison et al. 2009; Johnson et al. 2013; Lushchak et al. 2017). For instance, rapamycin treatment enhanced cognitive function of aged mice (Halloran et al. 2012) and reduced the proportion developing cardiac hypertrophy in mice (Shioi et al. 2003). Clinical trials of rapamycin and its analogs (rapalogs) targeting the mTOR signaling network have proved to be effective anticancer agents for several cancer types (Lamming et al. 2013; Meric-Bernstam and Gonzalez-Angulo 2009).

Like a CRM, rapamycin produces the healthspan-extending benefits similar to dietary restriction, but without requiring a reduction in food intake (Harrison et al. 2009). The investigators have shown feeding with rapamycin extends life span without reducing body weight even when treatment in middle age. However, rapamycin also generates serious side effects, particularly due to its immunosuppressive properties (Lamming et al. 2013; Mills et al. 2008), and thus it may not be suitable as an antiaging drug for humans. In spite of that, studies on mTOR signaling light up the road from basic discovery on the biology of aging to antiaging interventions.

15.4 Metformin

Metformin is firstly isolated from the French lilac and prescribed for anti-diabetes, attributing to its robust ability to suppress hepatic gluconeogenesis, to enhance peripheral glucose uptake, to decrease absorption of glucose from the gastrointestinal tract, and to increase fatty acid oxidation (Collier et al. 2006; Kim et al. 2008; Viollet et al. 2012). The mechanism of metformin action relies on the activation of the nutrient and energy sensor AMPK to inhibit expression of hepatic glyconeogenic genes (Collier et al. 2006; Kim et al. 2008) and to stimulate GLUT4 translocation to the plasma membrane to improve insulin-independent glucose uptake (Bailey and Turner 1996; Collier et al. 2006; Kim et al. 2008). Independent of AMPK

activity, metformin decreases hepatic gluconeogenesis by non-competitively inhibiting the redox shuttle enzyme mitochondrial glycerophosphate dehydrogenase to reduce the conversion of lactate and glycerol to glucose (Madiraju et al. 2014). Recently, metformin has been shown to regulate global DNA methylation to promote metabolic reprogramming (Cuyàs et al. 2018). Metformin increases life span in worms by activating AMPK/mTORC1-mediated autophagy (Chen et al. 2017) or mediating the microbial folate and methionine metabolism. However, in *Drosophila*, Slack et al. observed metformin is insufficient to prolong life span though AMPK was activated, even toxic when treated at high concentrates (Slack et al. 2012). Chronic metformin exposure extends the life span and healthspan of male middle-aged mice (Martin-Montalvo et al. 2013). Anisimov et al. have shown that metformin extends life span of cancer-prone mouse models (Anisimov et al. 2008). They also observed that metformin slightly promoted life span of female mice but decreased life span in male mice (Anisimov et al. 2010), yet it exerts no significant effects when feeding on normal rats (Smith et al. 2010). Another recent study suggests that the positive effects of metformin in mouse model of Alzheimer's disease are independent of AMPK (Kickstein et al. 2010). Additionally, many studies found the role of metformin in reducing incidence of age-related diseases, including cardiovascular diseases (Papanas and Maltezos 2009) and chronic kidney diseases (Pilmore 2010), supporting its potential as a CRM. However, unlike CR, metformin stimulates food intake (Anisimov et al. 2011; Martin-Montalvo et al. 2013). Scientists believe metformin is still at the top of the list of candidate CRMs, and research on the dose dependency of the effects on life extending and health promoting will broaden its clinical application range.

15.5 Sirtuin-Activating Compounds

Sirtuins (SIRT1–7 in mammals) are an evolutionarily conserved family of NAD^+-dependent deacylases with diverse subcellular distributions. Sirtuins play crucial roles in most biological processes, such as energy metabolism, stress response, genomic maintenance, cell proliferation, apoptosis, cancer, and aging. It is well-known that sirtuins serve as conserved longevity genes cross-species. BRASTO mice with ectopic SIRT1 in brain tissue (Satoh et al. 2013), male mice with additional copies of SIRT6, and mice treated with sirtuin-activating compounds (STACs) such as resveratrol and SRT2104 or NAD^+ precursors display increased life span expectancy. Sinclair's group has shown that resveratrol and other STACs extend life span of *C. elegans* and *Drosophila* by activating sirtuins, without reducing fecundity (Wood et al. 2004). Via high-throughput screening, compounds that activate SIRT1 have been largely identified, which can be classified into two groups: those of natural origin, which are phytochemical compounds (polyphenols) such as resveratrol, butein, quercetin, and myricetin, and nonrelated synthetic compounds. Of

note, despite the marked properties of quercetin and piceatannol on antioxidant and anti-inflammation, they did not produce significant effects on life span in more than three model organisms.

15.6 Resveratrol

Resveratrol (3,5,4′-trihydroxystilbene) is a natural polyphenol mainly found in grapes and berries (Nikolai et al. 2015). Its potential to promote life span and slow the general age-related decline has been examined in a wide variety of model organisms but first identified in yeast (Howitz et al. 2003). Resveratrol offers protection in models of stress- and age-associated diseases, including type 2 diabetes, neurodegeneration, and cardiovascular diseases (Baur et al. 2006; Lagouge et al. 2006). Long-term treatment of resveratrol remarkably improves cognitive abilities in elderly patients (Witte et al. 2014). Low dose of resveratrol in human $CD4^+$ T cells stimulates genotoxic stress and leads to the metabolic reprogramming, subsequently resulting in enhanced effector functions (Craveiro et al. 2017). In monkeys fed a diet high in sugar and fat, resveratrol supplementation attenuates the peripheral inflammation in adipose tissues (Jimenez-Gomez et al. 2013), maintains the pancreatic homeostasis by preventing β-cell dedifferentiation (Fiori et al. 2013), and improves vascular function, particularly pulse wave velocity (Mattison et al. 2014). The life span-extending benefits of resveratrol have been evidenced in flies (Bauer et al. 2004), worms (Morselli et al. 2010), fishes (Valenzano et al. 2006), and mice fed a high-fat diet (Baur et al. 2006). The mechanisms behind these effects may rely on the fact that resveratrol recapitulates CR-like effects in metabolic actions (Timmers et al. 2012). Resveratrol interacts with many stress-related targets in the cell, including NAD^+-dependent deacetylase SIRT1 (Baur and Sinclair 2006; Lagouge et al. 2006), which belongs to the sirtuin family that is linked to longevity in yeast, flies, and worms (Haigis and Guarente 2006; Kaeberlein et al. 1999; Mouchiroud et al. 2013).

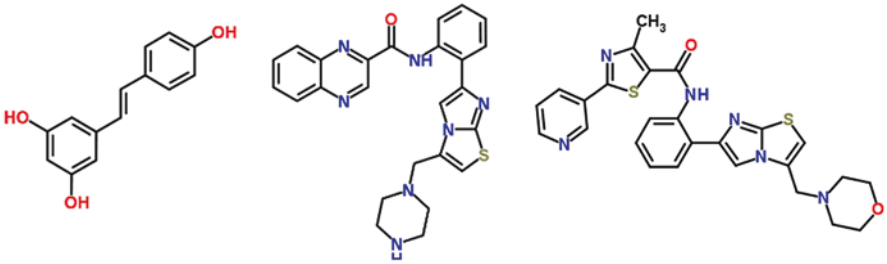
Overall, it is clear that resveratrol treatment postpones several age-associated diseases and pathogenic conditions, including oxidative stress in aging heart, neurodegeneration, or diabetes (Haigis and Guarente 2006). Based on meta-analysis of the available clinical data in treating type 2 diabetes, Hausenblas et al. conducted a comprehensive summary that the health benefits of resveratrol supplementation from human data are consistent with those from rodent studies (Hausenblas et al. 2015). Larger studies are needed to determine the bioavailability and dosage effects of resveratrol treatment in humans (Patel et al. 2011; Tome-Carneiro et al. 2013). More recently, a phase II study in patients with Alzheimer's disease suggested that resveratrol treatment was safe and well-tolerated and delay cognitive decline in the ability to perform daily tasks (Turner et al. 2015). However, it is worthy to note that several data claim that the degree of life span extension in worms and flies on a resveratrol-supplemented diet may be shorter than previously reported (Bass et al. 2007; Burnett et al. 2011). Resveratrol has failed to extend life span in mice fed on

normal diet (Baur et al. 2006; Pearson et al. 2008). Another study in healthy men showed that resveratrol had no effect on their physiological improvements (Poulsen et al. 2013). Recently, one clinical trial addressed daily oral supplementation of resveratrol had no beneficial improvement on men with metabolic syndrome (Kjaer et al. 2017). In some cases, resveratrol presents the detrimental effects in some types of cancers and in nonalcoholic fatty liver disease (Berman et al. 2017). Another study indicated that resveratrol in high dose and/or long-term treatment declined the antigen receptor signaling in human CD4+ T cells (Craveiro et al. 2017).

15.7 SRTs

SRTs, small SIRT1 activators including SRT2104 and SRT2104 structurally unrelated to resveratrol and with an EC50 in the low nanomolar, are currently evaluated as potential drugs for the treatment of T2DM, colitis, neurodegeneration, atherosclerosis, and other disorders. Compared to resveratrol, small molecule SIRT1 activators more effectively provided the improved insulin sensitivity and lower plasma glucose in obese mice (Milne et al. 2007). SRT1720, thousand-fold more potent to activate SIRT1 than resveratrol, extends the life span of adult mice fed a high-fat diet or a standard diet, accompanied with general health benefits including improved metabolism and reduced inflammation (Minor et al. 2011; Mitchell et al. 2014; Yamazaki et al. 2009). Milne et al. demonstrated SRT1720 allosterically regulates SIRT1 activity and improves glucose homeostasis and insulin sensitivity in diet-induced obese and genetically obese mice and Zucker fa/fa rats (Milne et al. 2007). SRT1720 induces mitochondrial biogenesis and respiration via SIRT1-targeted PGC-1α deacetylation instead of AMPK signaling (Funk et al. 2010). In the preclinical evaluation of cancer treatment, SRT1720 significantly inhibits tumor growth and induces cell death in multiple myeloma cells and breast cancer cells (Chauhan et al. 2011; Lahusen and Deng 2015). SRT1720 alleviates cholesteric liver injury in mice fed a cholic acid diet by activating SIRT1 (Kulkarni et al. 2016). Despite of so many promising benefits from its pharmacological data, the clinical trials of SRT1720 on humans have not been performed yet. SRT2104, a first-in-class and highly selective small molecule activating SIRT1, has been shown to extend the mean and maximal life span in mice fed a normal diet, accompanied with improved whole-body physiology (Mercken et al. 2014). Repeated oral administration of SRT2104 in multiple phase I trials demonstrated both safety and bioavailability in healthy volunteers (Hoffmann et al. 2013). Two clinical trials for SRT2104 in elderly volunteers and otherwise healthy cigarette smokers showed an increased glucose tolerance and improved serum lipid profile (Libri et al. 2012; Venkatasubramanian et al. 2013). A separate trial to study the therapeutic approach for patients with an inflammatory disease such as psoriasis displayed an excellent histological improvement in the group of oral administration of 500 or 1000 mg per kg SRT2104 (Krueger et al. 2015). In another study, the effects of oral doses of SRT2104 on anti-inflammatory were determined in whole blood of healthy adult subjects (van der Meer et al. 2015). However, one recent phase II study in adults

with T2DM showed 28-day treatment of SRT2104 had no improvement of glucose or insulin (Baksi et al. 2014). Thus, there is still a long road ahead.



15.8 NAD⁺ Precursors

NAD^+, as a cofactor for numerous enzymes, is critical for cellular energy metabolism, DNA repair, and adaptive responses to bioenergetic and oxidative stress. Compromised NAD^+ status has been shown in various tissues of aging or premature aging animal models, linking NAD^+ depletion to multiple hallmarks of aging (Fang et al. 2017). The molecules to restore the NAD^+ levels have been largely discovered, including NAD^+ precursors, nicotinamide (NAM/vitamin B3), nicotinic acid (NA), tryptophan (Trp), nicotinamide riboside (NR), and nicotinamide mononucleotide (NMN) (Verdin 2015), and inhibitors of CD38 (glycohydrolases that degrade NAD^+) such as flavonoid apigenin, quercetin, and compound 78c (Escande et al. 2013; Haffner et al. 2015). NAD^+ replenishment extends life span and healthspan in yeast, *C. elegans*, *Drosophila*, and mice. NR treatment extends the average life span of worms in SIR-2.1-dependent manner (Fang et al. 2016). Overexpression of NAD^+ synthetic enzyme nicotinamidase (D-NAAM) in *Drosophila*, improving the NAD^+ salvage pathway, increases both mean and maximal life span by up to 30% via a Sir2 pathway (Balan et al. 2008). NR supplementation in 2-year-old C57BL/6 J mice significantly increases longevity (Zhang et al. 2016). Restoration of NAD^+ improves the life span and healthspan in *C. elegans* models of XPA and CS (Fang et al. 2014; Scheibye-Knudsen et al. 2014), which exhibit severe premature aging features primarily caused by DNA repair impairment. A recent literature addressed that NR administration extends the life span of *Atm−/−* mice and *atm-1* mutant worms (Fang et al. 2016). NMN treatment increases the life span of BubR1 (a mitotic checkpoint kinase) mice (North et al. 2014). Boosting the NAD^+ system with NA in human peripheral blood mononuclear cells improves genomic stability (Weidele et al. 2017).

Supplementation with NAD^+ precursors not only extends life span but also improves healthspan in yeast, flies, worms, and mice, as shown by improved mitochondrial health, muscle strength, and motor function. Feeding with NR in mice

enhances oxidative metabolism and protects against glucose dysregulation induced by high-fat diet (Canto et al. 2012), restores muscle mass (Frederick et al. 2016), and prevents DNA damage and carcinogenesis (Tummala et al. 2014), noise-induced hearing loss (Brown et al. 2014), heart failure (Xu et al. 2015), and steatosis (Mukherjee et al. 2017). Vitamin B3 supplement prevents glaucoma in aged mice by restoration of mitochondrial function (Williams et al. 2017). Treatment with the related molecule NMN protects diet- and age-induced T2DM by enhancing mitochondrial function and insulin sensitivity in muscle and the liver, in a SIRT1-dependent way (Yoshino et al. 2011).

In summary, NAD^+ precursors have been shown to improve life span and healthspan in normal and prematurely aged laboratory organisms, but whether these findings can be translated to humans remains unproven and requires further investigation. Trammell et al. tested the oral availability and utilization of three NAD^+ precursors, i.e., NR, NAM, and NA, in mice and humans. NR supplementation uniquely and safely increases the blood NAD^+ metabolism, rendering the enabled clinical trials of NR to improve health in humans (Trammell et al. 2016). With careful scientific evaluation and validation, NAD^+ precursors may serve as promising candidates to slow aging and improve the quality of life in humans.

15.9 Autophagy Inductors

Autophagy, an evolutionarily conserved process, is cytoprotective to remove long-lived, unfolded, or damaged proteins. Increasing lines of evidence suggest that autophagy is required for organismal development and health. During aging, protein homeostasis declines and damage accumulates. Indeed, autophagic rates decline over time in most organisms, and autophagic dysfunction is associated with many age-related pathologies, like Parkinson's and Alzheimer's diseases (Li et al. 2017; Rivero-Rios et al. 2016). Many strategies that interrupt aging process, such as CR, are molecularly involved in regulating autophagic activity in various organisms. Autophagy is crucial for life span extension by reduced germline, inhibition of insulin/insulin growth factor signaling, or TOR signaling (Rubinsztein et al. 2011). Melendez et al. observed that loss-of-function mutation in the insulin-like growth factor pathway activates autophagy in *C. elegans*, and inhibition of autophagy by mutating essential *Atg* genes prevents life span extension, indicating that increased autophagy contributes to longevity (Melendez et al. 2003). The pharmacological activators of autophagy to extend life span and healthspan have been evaluated, for example, rapamycin and spermidine.

15.10 Spermidine

Spermidine, a naturally occurring polyamine that triggers autophagy by inhibiting the acetylation status of histone to upregulate the expression of *Atg* genes, extends life span in multiple laboratory organisms. Endogenous spermidine concentrations

significantly decrease as humans age with the exception of centenarians (Pucciarelli et al. 2012). Supplementations of spermidine activate autophagy independent of SIRT1 in human and yeast cells as well as worms (Morselli et al. 2011), thus promoting healthspan of yeast, flies, and worms (Eisenberg et al. 2009). Long-term administration of spermidine extends life span and exerts cardioprotective effects in old mice (Eisenberg et al. 2016). Lifelong administration of spermidine reduces liver fibrosis and hepatocellular carcinoma and increases the life span up to 25% (Yue et al. 2017). Life span promotion has been reported by upregulation of spermidine intake or gut polyamine produced by bacteria in two short-lived mouse models (Matsumoto et al. 2011; Soda et al. 2009). Additionally, spermidine has been found to induce neuronal autophagy and impede a number of neurological pathologies. Spermidine prevents neuronal cell damage by inducing autophagy via blocking caspase 3-mediated Beclin 1 cleavage (Yang et al. 2017). Moreover, age-associated memory decline in flies is rescued by spermidine in an autophagy-dependent fashion (Gupta et al. 2013; Sigrist et al. 2014). Surprisingly, Wilhelm et al. recently demonstrated the opposing effects of autophagy on longevity, showing that inactivation of genes governing early stages of autophagy, such as Atg6/beclin1 ortholog *bec-1*, strongly extends both life span and healthspan in *C. elegans* (Wilhelm et al. 2017).

15.11 Senolytics

Cellular senescence, driven by various genotoxic, oxidative, and inflammatory stress, has been demonstrated to be a key mediator of aging (Tchkonia et al. 2013). In 2011, a study found that eliminating $p16^{Ink4a}$-positive senescent cells delays the onset of many age-related pathologies, implicating clearance of senescent cells can extend healthspan (Baker et al. 2011). Thereafter, dozens of experiments have confirmed that clearing the accumulated senescent cells alleviates or prevents certain age-related dysfunctions. In early 2015, the first senolytics were identified, i.e., dasatinib and quercetin, which in combination can effectively eliminate the senescent cells and alleviate a range of age-related disorders in mice (Zhu et al. 2015). Another senolytic compound, ABT263 (now known as navitoclax), has been discovered to inhibit BCL-2 and BCL-xL that promote the cells to survive by several independent laboratories (Chang et al. 2016a; Yosef et al. 2016; Zhu et al. 2016). But it exerts cell type-specific, being effective to trigger apoptosis in HUVECs but not preadipocytes (Chang et al. 2016b). When performing senolytics' tests on normally aged mice, Baker et al. showed that killing off senescent cells by AP20187 treatment in 1-year-old mice delays the age-related deterioration of various organs, including the kidney and heart, and fat. Notably, it extends the animals' median life span by about 25% (Baker et al. 2016). In addition, a peptide that activates a cell death pathway restores lustrous hair and physical fitness in aged mice (Baar et al. 2017). It is conceivable that many more senolytic drugs will emerge over the next few years. Though many prestigious investigators express more optimism on the senolytics as the pharmacological approach to improve human healthspan, the safety and efficacy of these drugs before routine clinical use should be carefully and fully evaluated.

15.12 Conclusion

The increased percentage of elderly people, with the morbidity of age-related diseases such as T2DM, heart diseases, cancers, and neurodegenerative diseases, is projected to increase substantial healthcare costs in the coming decades. Aging is a rather complex process. As the findings from model organisms revealed, aging process is plastic to be manipulated by both genetic and environmental factors. Manipulations on aging-related genes by diet, lifestyle, and pharmaceuticals are able to dramatically improve life span and healthspan. One well-studied dietary manipulation of aging is CR, limiting food intake but without inducing malnutrition. However, fasting and exercise regimens seem hard to be followed worldwide. A large number of small molecules targeting aging process have emerged to prolong life span and/or sustain health in experimental animals. These interventions are all compatible within the molecular mechanisms by modulating critical components and pathways responsible for the aging program, including mTOR signaling, sirtuins, AMPK, autophagy, IGF1 signaling, cellular senescence, etc. As a consequence, the improved cellular functions, such as mitochondrial efficiency and stress resistance, hold off the aging-associated physiological declines and pathological manifestations. Most investigators realize that greater understanding of the molecular mechanisms involved in aging process basically promotes identification and translation of the extrinsic solutions to slow the rate of aging and delay the onset of age-related diseases. Developing pharmacological agents as candidates for antiaging drugs is now in the spotlight in "geroscience."

However, there are still many barriers when translating to human aging. In principle, aging cannot be quantified, and clinical trials running on humans cannot be performed. Though human life span can be recorded, as well as health monitors such as blood pressure, insulin sensitivity, lipid profile, inflammation, nutritional determinants, etc., these data may or may not represent as the biomarkers of aging (Mallikarjun and Swift 2016). Another issue is safety and bioavailability when long-term interventions are performed on humans (Kirkland and Peterson 2009). Although testing compounds directly for effects on human aging are not realistic at present, the clinical application with some compounds, such as rapamycin and metformin, may eventually be shown to extend healthy life span, when used in older individuals. Clearly, breakthroughs in the biology of aging and unlocking the capacity to manipulate human aging would result in unprecedented health benefits. Many researches and biotech companies are more inclined to focus on assessment and validation of the potential therapeutic effects on aging-related disease in humans.

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Application of Stem Cell Technology in Antiaging and Aging-Related Diseases

16

Yanqiu Yu

Abstract

Stem cells are one kind of cells that have the potential of proliferation and differentiation. The human beings are originated from a totipotential stem cell—fertilized egg. After birth, the proliferation and differentiation of stem cells contribute to the development and maturation of individual tissues and organs. After maturation, aging is a phases of the life process, the stem cells within the individual's tissues ensure the metabolism of different cells and tissues, such as the hematopoietic stem cells in the bone marrow, which ensure there are still enough red blood cells (RBCs) being responsible for the mission of transporting oxygen after a single RBC has completed its 120-day physiological life cycle. After pathological damage and necrosis occurring on the intestinal epithelial cells or tubular epithelial cells, there will be regenerative epithelial cells continuing to maintain the integrity of the structure and function of the intestine and renal tubules. The role of stem cells in the regeneration and repair of tissues and organs is not only because of the ability of proliferation and differentiation of stem cells but also of the secretion function of stem cells, which secrete various growth factors and cytokines to regulate the tissue microenvironment. For example, mesenchymal stem cells derived from bone marrow are important regulators in bone marrow hematopoietic stem cell niche. Mesenchymal stem cells maintain the "stemness" of hematopoietic stem cells by secreting various cytokines.

Aging is a phases of the life process, and all creatures obey this rule of nature. Different organs of the body have different time of entering into aging. Aging is reflected in structural changes and reduced function. Among them, the reduction of regeneration and repair capacity is the main feature of aging. As we age, the

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aging of stem cells in human tissues is a major cause of the decline in tissue regeneration capacity. Therefore, the elderly's ability of regenerate and repair can be improved by application of advance stem cell technology. It can delay the aging process and treat aged diseases (showed in Fig. 16.1).

Keywords
Stem cell · Aging-related diseases · Clinical trials · Cellular drugs

16.1 The Development of Stem Cell Technology

Since 1998, Thomson J.A and others reported the establishment of the human embryonic stem cell line for the first time in "science," stem cells have triggered a sensation in the scientific community and led to the development of biotechnology, biomedicine, and clinical treatment technologies (Baharvand 2009) (Fig. 16.1).

16.1.1 Stem Cell Transplantation Technology

Stem cell technology is applied in many clinical disciplines (Duncan and Valenzuela 2017). The earliest application of stem cell technology is cell transplantation technology, which is currently the most widely used in clinical practices (Gnecchi and Cervio 2013). In 1945, Dr. Lorenz from the United States applied hematopoietic stem cell suspension to treat the hematological disease caused by lethal doses of radiation on mice in order to find an effective treatment for patients with blood diseases caused by the atomic bomb in Hiroshima of Japan during World War II. In 1968, the world's first clinical bone marrow transplantation gained success, which opened the curtain for the cell transplantation technology which uses the hematopoietic stem cell transplantation for the treatment of certain hematological malignancies and tumors. In 1988, Professor Gluckman from France used cord blood-derived hematopoietic stem cell transplantation to successfully treat an anemic child and started cord blood stem cell transplantation. In 1992, the first umbilical stem cell blood bank in the United States was established in New York. In 1996, the first cord blood stem cells were delivered in the form of "cellular products," and the industrialization of "cellular products" was initiated. In 2009, the first bone marrow-derived mesenchymal stem cell product, "Prochymal," was licensed by the FDA organization in Canada and New Zealand. The allogeneic mesenchymal stem cell product is applied by a cell transplantation technique to treat serious graft-versus-host disease (GVHD) after bone marrow transplantation. This marks the official "cellular drug" status of stem cells as a clinical cell therapy technology.

16.1.2 Stem Cell Technology and Transgenic Technology

Because stem cells have a strong proliferative and differentiative ability in vitro, they, therefore, have become target cells for some gene therapy. Researchers can use transgenic techniques to correct some stem cells with gene deletion and gene mutation and then induce the corrected stem cells to differentiate into normal tissue cells. Such as sickle cell anemia, the researchers genetically repair hematopoietic stem cells in patients with sickle cell anemia or transform epithelial cells into induced pluripotent stem cells (iPSCs) and perform gene correction and then use the corrected stem cells to transplant for the treatment of the hereditary diseases.

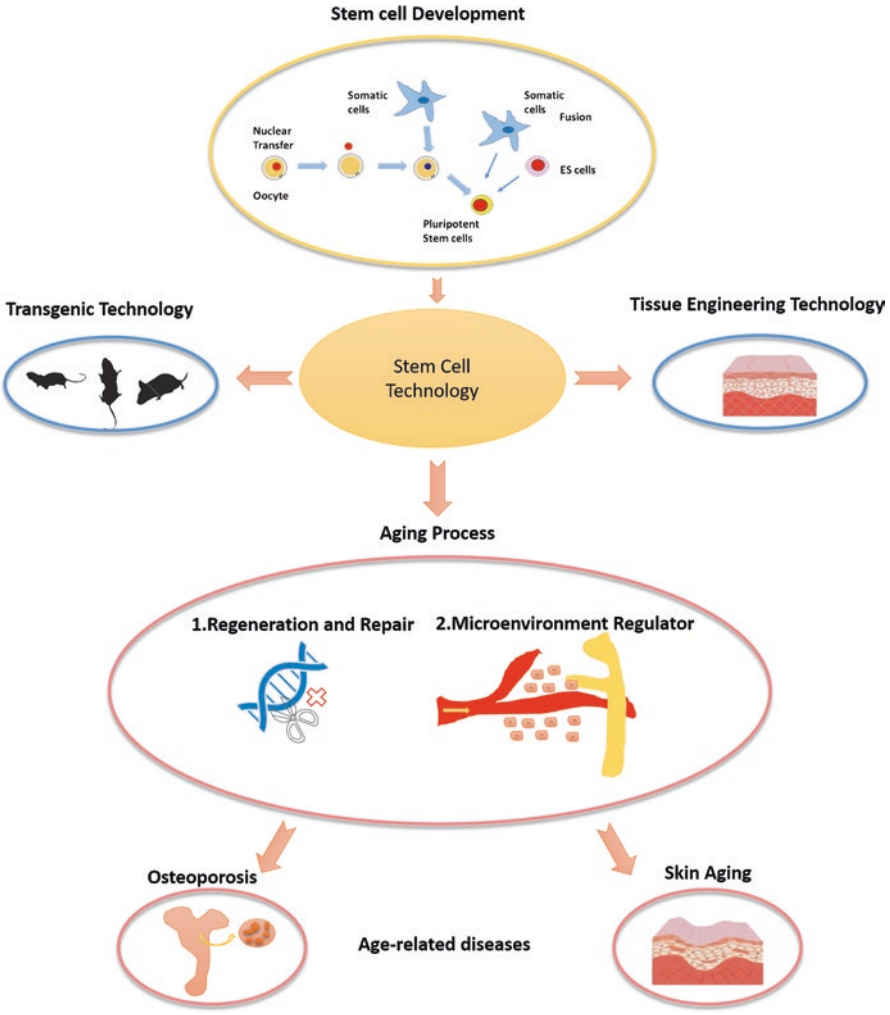

Fig. 16.1 Application of stem cell technology in antiaging and aged diseases

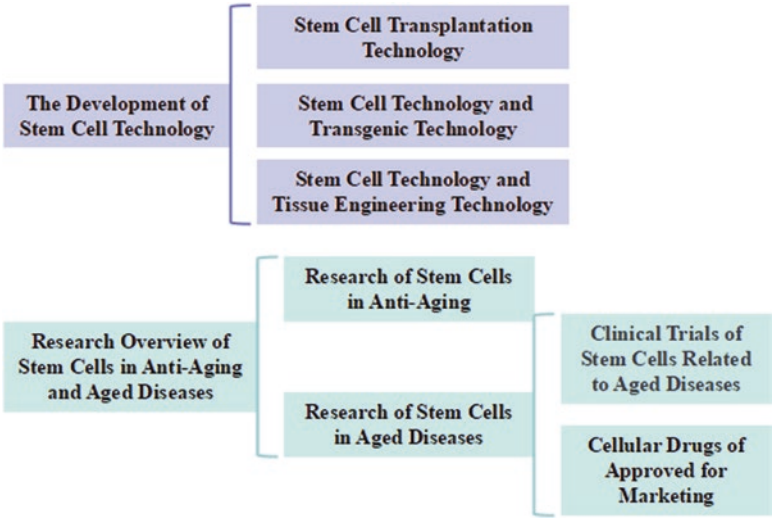

Fig. 16.1 (continued)

Japanese researchers reported that obtaining induced pluripotent stem cells by transgenic technology and producing large numbers of red blood cells in vitro to achieve "artificial blood cells" provide a cellular source for cell transplantation in patients with diseases of the blood system. Using autologous differentiated cells, inducing them into iPSCs by transgenic technology, then differentiate into different cells according to clinical disease treatment needs, and perform cell transplantation. This combination of stem cells and transgenic technology addresses the cellular source problems of cell transplants, especially those cells that need to be committed, such as bone marrow hematopoietic stem cells. Using autologous induced stem cells by transgenic technology to solve the cellular source problem of the clinical cell transplantation has become a hotspot in clinical research; researchers are trying to find a safe and effective application program for iPSCs in the clinical practice, especially for those elderly, or elderly patients. iPSCs can achieve the goal of "rejuvenating the aged" at the cellular level and provide a good treatment plan for tissue and organ repair in elderly patients.

The previous chapters of this book also introduce that as human beings grow, somatic cells of all tissues enter into the aging process. Although the speed and manifestation of cells in different tissues are different, the common characteristic of aging is the decline in proliferation and differentiation capacity, which is due to the decrease in cellular proliferation capacity and shortening of telomerase with the proliferation algebra. Therefore, the ability of the elderly to repair tissue damage decreased due to reduced proliferation and differentiation ability of stem cells. Stem cell technology combine with transgenic technology can restore human cells to the embryonic state which has proliferative and differentiation potential, such as iPSCs, reversing the aging process and providing an effective strategy for human antiaging.

16.1.3 Stem Cell Technology and Tissue Engineering Technology

Tissue engineering is an emerging discipline that combines the cell biology and materials science for the in vitro or in vivo construction of tissues or organs. Cells are an essential element in tissue engineering technology. Stem cells become the best seed cells for tissue engineering because of their ability to proliferate and differentiate in vitro. The different functional cells will be derived from stem cells by induced culture, resolving the problem of cellular source in tissue engineering, for example, induction of embryonic stem cell in vitro to differentiate into nerve cell, which can be used as the nerve cell constituting the nerve fiber in tissue engineering. Hepatocytes differentiated from embryonic stem cells can become seed cells of "mini-liver." With the development of biomaterials, stem cells, and tissue microarray technology, human beings will create the "true organs" of the human body in vitro and provide the patients with tissue-engineered organs for replacement, such as transplantation of tissue-engineered skin and blood vessels. For those with senile vascular disease, it is expected that the use of tissue-engineered blood vessels will replace those with severe atherosclerotic lesions or infarct vessels, thereby improving tissue supply and organ function, such as myocardial infarction, cerebral infarction, lower extremity arterial thrombosis, etc.

16.2 Research Overview of Stem Cells in Antiaging and Aged Diseases

Aged disease, also named as geriatric disease, refers to the aging-related diseases and is generally divided into specific diseases of the elderly and high incidence of senile patient. Senescence is the causative factor of aged diseases. Senescence leads to the structural change of various organs and the decline of functions such as Alzheimer's disease and senile deafness. Under physiological conditions, different cells will obey their respective life cycles, from the proliferation and differentiation, mature to aging. Senescent cells in the tissue are removed in time and replaced by regenerative cells. With age, the ability to maintain the number and functional cellular homeostasis decreased, self-repairing capability of aged patient decreased, which is also one of the factors that leads to high prevalence of some diseases in the elderly, such as arterial atherosclerosis.

Adult stem cells are the main determinants of tissue regeneration and repair. The ability of stem cells to proliferate and differentiate can reflect the self-repairing capability of tissues and organs (Tsang 2013). Therefore, stem cells are often used as research objects or to replicate cellular senescence models. At the cellular level, aging mechanisms are studied, and the effect of various substances on aging is examined. For example, we use the skin stem cells to observe the effect of ultraviolet radiation on the proliferation, differentiation, and secretion function of skin stem cells to explore the pathogenesis of photoaging. Meanwhile, we also use conditional medium of stem cell and exosomes derived from stem cells to explore the mechanism of stem cell anti-photoaging.

Therefore, in the study of aging and aged diseases, stem cells are a good model of aging mechanism and also an antiaging method. Supported by a variety of laboratory research data, researchers from all over the world started to study the clinical application of stem cells.

The main mechanisms of stem cells' antiaging and the treatment of aged diseases are as follows:

1. The function of regeneration and repair. The stem cells proliferate and differentiate into different functional cells to replace aging and damaged cells (Stamm et al. 2009), maintaining the integrated structure and normal function of tissues and organs (Eirin and Lerman 2014; Somasundaram 2014).
2. Microenvironment regulators. Stem cells can secrete various biologically active proteins, including growth factors, cytokines, and the like, and provide biological functions of different cells in tissues and organs through an intercellular signal transduction system (Galkowski et al. 2017). For example, mesenchymal stem cells (MSCs) can secrete endothelial growth factor, initiating microvascular endothelial progenitor cell proliferation and microvascular regeneration (Somasundaram 2016); MSCs can also secrete cytokines that regulate the immune function, such as the first drug for the treatment of GVHD; and MSCs secrete a variety of extracellular matrix proteins, improving the microenvironment of the tissue (Shevela et al. 2016) and maintaining the "stemness" of stem cell niche and the body's ability to regenerate and repair (Liu 2013).

16.2.1 Research of Stem Cells in Antiaging

The histological character and function of human organs change with age. The most obvious one of all is facial aging. Another obvious change with age is the body's motor system, the most important of which is the human skeletal system. The balance between osteoblast and osteoclast is broken with age, and osteoporosis and osteoarthritis become the obvious feature of aging, which lead to function decline of motor system in the elderly. We take facial aging and osteoarthritis as the examples to explore the application of stem cells in antiaging.

Facial aging is a complex process that affects both 3D shape and textures, such as dryness, coarseness, lost luster, and wrinkles. We analyzed the conditional medium which is used to culture cord Wharton's jelly-derived stem cells, placenta-derived stem cells (PSCs), and adipose-derived stem cells (ASCs). As Meenakshi Gaur reported (Yan et al. 2016), the specific proteins secreted from ASCs were more adept at cell adhesion, migration, wound healing, and tissue remodeling, while the proteins secreted by PSCs were more adept at angiogenesis, cell proliferation, differentiation, cell survival, immunomodulation, and collagen degradation. While the conditioned medium (CM) could improve the facial index, the improvement of melanin index after injection of the adipose stem cell-conditioned medium (ASC-CM) was much more significant.

The adipose-derived stem cells are most commonly used in plastic surgery as seed cells. The ASC-CM has also been proven to play an important role in the prevention of photoaging dermal cells. In our study, we found the facial mask made in adipose stem cell-conditioned medium, which could promote skin repair and regeneration. The clinical trials (Gaur et al. 2017) showed that ASC-CM and PSC-CM were injected into facial skin and greatly improved the facial indexes, such as erythema and melanin. ASC-CM and PSC-CM were only different in the index of melanin. The secretory proteins in CM were detected and that TGF-β was an essential protein in antiaging, the mechanism of antiaging still requires further inquiry. The results from a lot of laboratories suggest that stem cells and secreted proteins are ideal cells and cell-free substances for regeneration medicine, especially in the antiaging field.

Osteoarthritis (OA) is one of the most common debilitating disorders among the elderly population, which is one of aged disease. At present, there is no definite cure for the underlying causes of OA. ASCs were confirmed those could regenerate cartilage. In some clinical trials, ASCs, no-culture-expanded, in the form of stromal vascular fraction (SVF) along with platelet-rich plasma (PRP), have recently been used in humans to treat OA and other cartilage abnormalities. These ASCs have demonstrated effectiveness without any serious side effects. However, due to regulatory issues, only ASCs in the form of SVF are currently allowed for clinical uses in humans. Culture-expanded ASCs, although more convenient, require clinical trials for a regulatory approval prior to uses in clinical settings. The clinical studies showed that intra-articular injection of ASCs in the form of SVF with PRP or without PRP was safe and efficacious in treating OA. Moreover, obtaining approximately 100 g of adipose tissue and percutaneous joint injections is considered to be a minimally invasive procedure and can be readily accepted by patients. These procedures carry relatively low rates of morbidity and side effects (Pak et al. 2016). A large number of papers have shown that ASCs in the form of SVF with PRP can be efficacious in symptom improvement, but it still needs more clinical trials to explain the mechanism of ASCs in treating OA.

The data like Dajeong Kim (Kim et al. 2015; Zhang et al. 2015) reported, they transplanted human amniotic membrane-derived mesenchymal stem cells or adipose tissue-derived mesenchymal stem cells (1×10^6 cells per rat) to 10-month-old male F344 rats once a month throughout their lives. Transplantation of stem cells improved cognitive and physical functions of naturally aging rats, extending life span by 23.4% and 31.3%, respectively. The stem cell therapy elongated both health span and life span, which could be a starting point for antiaging or rejuvenation effects of allogeneic or autologous stem cells with minimum immune rejection.

At present, researchers in the world are trying to explore the mechanism and know-how of stem cells in antiaging.

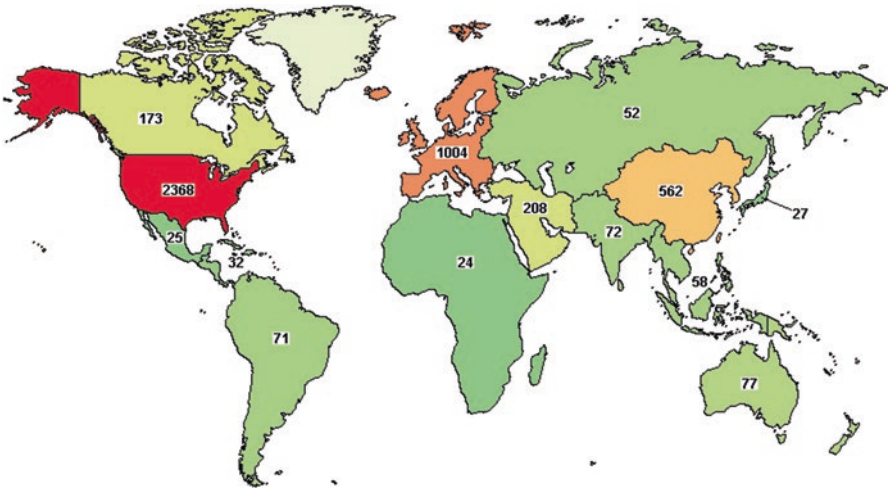

Fig. 16.2 4471 studies found for stem cells on https://www.clinicaltrials.gov/. The data was updated to May 7, 2017, "stem cell" as index words

16.2.2 Research of Stem Cells in Aged Diseases

The data displayed on https://www.clinicaltrials.gov/ ("stem cell" as index words, data updated to 05-07-2017) showed that 4471 "stem cell"-related clinical trials were registered (showed in Fig. 16.2). And 3939 clinical trials of stem cells are found to be related to aged diseases (showed as Fig. 16.3), including coronary heart disease, cardiac failure, stroke, Parkinson disease, Alzheimer's disease, osteoporosis, and so on.

In the 3939 clinical trials, there are 39% of clinical trials completed. As Fig. 16.4 A showed, 21% of clinical trials are recruiting now. Three percent of clinical trials are not yet recruiting. Thirty-seven percent of clinical trials are in clinical process. And 4% of those are IV phase, 10% of those are III phase, 45% of those are II phase, and 41% of those are I phase.

In fact, these are not all the stem cell-related clinical trials registered on https://www.clinicaltrials.gov/. There are more clinical trials than those showed on this web. It is difficult to gain the accurate data on stem cell-related clinical trials in the world because the regulations on stem cell are different in different countries.

Since 2009, the stem cells as "cellular drugs," also named as the "living drugs," are applied in clinic. In the world, showed in Table 16.1, eight kinds of stem cell drugs are used to treat aged diseases.

With the development of stem cell technology and rapidly updated clinical data (Liau et al. 2016), the stem cell technology will become an effective treatment for aging-related diseases (Chase and Vemuri 2013) and the imaginative pathways and tools for antiaging.

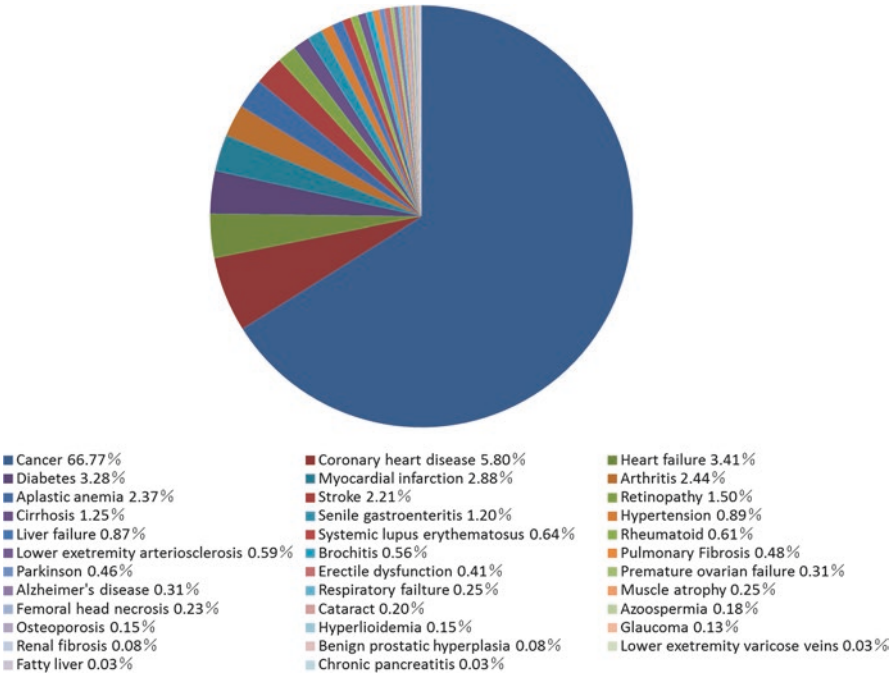

Fig. 16.3 The proportion of aged diseases in stem cell research. 3939 clinical trials of stem cells related to aged diseases on https://www.clinicaltrials.gov/ (updated to 07,05,2017)

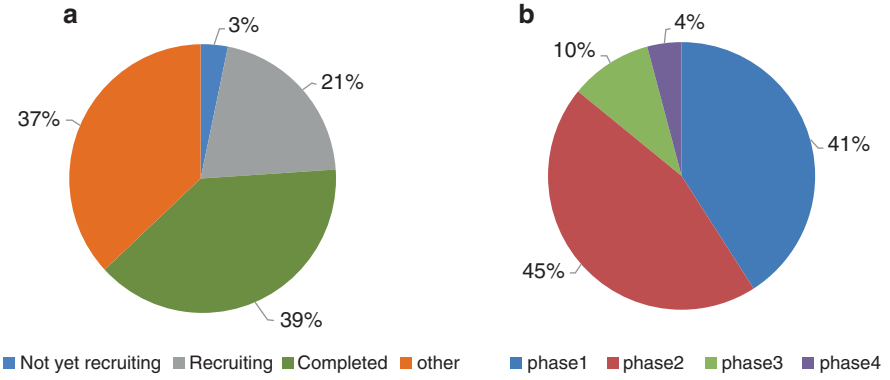

Fig. 16.4 The status of 3939 clinical trials of stem cells related to aged diseases on https://www.clinicaltrials.gov/ (updated to 07,05,2017)

Table 16.1 Cellular drugs of approved for marketing

Year	Nation	Name of medicine (company)	Cellular origin	Indications
2009.10	Belgium	ChondroCelect (TiGenix)	Autologous chondrocytes	Knee articular cartilage defect
2009.12	The USA	Prochymal (Osiris)	Human allogeneic bone marrow-derived mesenchymal stem cells	Type I diabetes
2010.07	Australia	MPC (Mescoblast)	Autologous mesenchymal precursor cells	Acute myocardial infarction
2011.07	South Korea	Hearticellgram-AMI (FCB-Pharmicell)	Autologous bone marrow mesenchymal stem cells	Acute myocardial infarction
2011.11	The USA	Hemacord (New York Blood Center)	Cord blood hematopoietic progenitor cells for allogeneic hematopoietic stem cell transplantation	Hereditary or acquired hematopoietic system disease
2012.01	South Korea	Cartistem (Medipost)	Allogeneic cord blood mesenchymal stem cells	Cartilage injury and degenerative joint diseases
2012.01	South Korea	Cupistem (Anterogen)	Autologous adipose-derived mesenchymal stem cells	Anal fistula
2012.05	Canada	Prochymal (Osiris Therapeutics)	Allogeneic bone marrow mesenchymal stem cells	Refractory childhood graft-versus-host disease (GVHD)
2015.02	Italy	Holoclar (Chiesi)	Ex vivo expansion of human autologous corneal epithelial cells containing stem cells	Moderate to severe limbal stem cell defect (LSCD)
2016.02	Japan	Temcell (JCR)	Allogeneic mesenchymal stem cells	Graft-versus-host disease (GVHD)

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Which is the Most Reasonable Anti-aging Strategy: Meta-analysis

17

Yaru Liang and Zhao Wang

Abstract

An organism's lifespan is inevitably accompanied by the aging process, which involves functional decline, a steady increase of a plethora of chronic diseases, and ultimately death. Thus, it has been an ongoing dream of mankind to improve health span and extend the lifespan. In the last century, there is a great increase in the search for eternal youth and an insatiable appetite for methods which could turn back the clock. Survival curves are key components of lifespan experiments. Many interventions have been reported to extend the lifespan, including the administration of pharmaceuticals, calorie restriction, and genetic alteration. However, few studies have attempted to provide a comprehensive analysis of the mechanism by which these various methods function to extend lifespan. We recently collected survival curves from published papers and recovered data by fitting models. The analysis results highlight the overall advantage of calorie restriction and its mimetics in aging and demonstrate that hypoglycemic agents and antioxidants have a superior effect on lifespan extension via a pattern of global integrity compared to other medications. This review provides a scientific foundation for the discovery of effective anti-aging agents and the formulation of scientific anti-aging strategies.

Keywords

Survival curve · Meta-analysis · Calorie restriction · Aging and anti-aging

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17.1 Introduction

Aging is a complex multifactorial process of molecular and cellular decline that affects tissue function over time, rendering organisms frail and susceptible to disease (such as arthritis, heart disease, osteoporosis, cancer, Alzheimer's disease, etc.) and death. Human beings and members of other species, especially animals, necessarily experience aging and mortality. Although many theories abound about the underlying mechanisms of aging, none of them can fully explain the process. Some maintain that aging is preprogrammed into our cells, while others contend that aging is primarily the result of environmental damage to cells (Kirkwood 2005). Here are some most popular aging theories, including the free radical theory, the telomere shorten theory, the DNA damage theory, the immune system theory, and so on.

The free radical theory of aging was conceived by Denham Harman in the 1950s (Harman 1956), which states that reactive oxygen species (ROS), extremely reactive chemical molecules, are the major cause of aging process. Free radicals are mainly produced by the mitochondrial respiratory chain as a result of electron transport and the reduction of the oxygen molecule, causing damage to certain macromolecules including lipids, proteins, and most importantly mitochondrial DNA. And the theory was modified by defining the mitochondrial respiration as the major cellular source of ROS and got the new name mitochondrial theory of aging (Harman 1972). Numerous studies reported that ROS and oxidative damage increase with aging (Stadtman 1992) and that reducing oxidative damage can extend lifespan of several model organisms, including yeast, nematodes, fruit flies, mice, and so on, while increased production of ROS shortens lifespan (Kirkwood and Kowald 2012). However, recently some workers in this field reported that superoxide radical and ROS exert beneficial effects (Fig. 17.1). For example, oxidative stress was reported to provoke longevity and metabolic health, which might be the result of mitochondrial hormesis or mitohormesis. By promoting an increase in the ROS and an increase in mild oxidative damage, one can foster stress defenses in mitochondria and increase metabolic health and lifespan (Ristow and Zarse 2010). Collectively, these studies argue against the universal role of oxidative damage in aging. Many researchers turned to a broader concept that many forms of damage serve as causal factors in the aging process, with ROS representing some of the major causes, but not the only cause. Oxidative stress is toxic because it causes increased damage to important cellular targets, increased mutation rate, growth inhibition, etc. At the same time and because of this, it decreases the metabolic rate and increases cellular generation time, whereas in turn decreased metabolism leads to decreased production of free radicals such as superoxide (Gardner 1997).

Telomeres are complexes composed of proteins and nucleotides of TTAGGG repeats at the ends of eukaryotic chromosomes, protecting the DNA when a cell divides. During replication, telomeres lose some of their genetic material but are repaired by the ribonucleoprotein telomerase. Due to the mechanism of replication, telomeres shorten with every cell division, ticking as a rather elegant biomarker used to indicate cellular senescence. Both telomeres and telomerase are associated

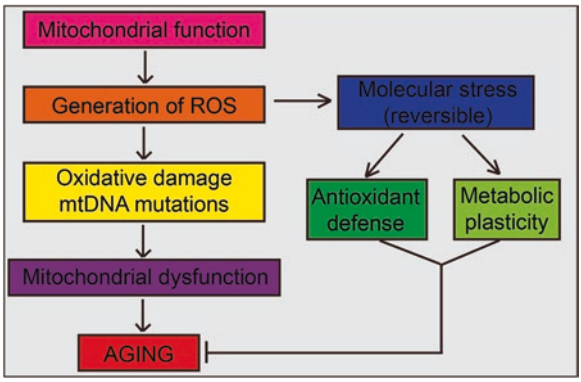

Fig. 17.1 The bidirectional effect of free radicals on aging. When radicals cause severe effects on biomolecules, it causes irreversible damage, whereas when the aggression is mild, a stress is caused, and this may have signaling effects, as well as hermetic effects

with cell senescence and apoptosis, and they play key roles in aging, cancer, hereditary syndromes, and chronic diseases (Zanni and Wick 2011). Telomere theory of aging is based on this telomere-shortening mechanism (Olovnikov 1973, 1996; Greider and Blackburn 1987). It indicates that aging is programmed and irreversible cell cycle arrest happens in response to the telomere shortening, and the total number of cell divisions in the absence of telomerase activity cannot exceed a particular limit termed the Hayflick limit (Olovnikov 1973; Greider and Blackburn 1987; Hayflick and Moorhead 1961). Nevertheless, it was reported that telomere length is an individual characteristic (Takubo et al. 2002) and can be dynamically changed throughout an individual's life period in response to environmental factors and stress (Baird et al. 2011; Aviv 2002; Carlson et al. 2014). Though these data seem to be contradictory to telomere theory, it is for the reason that some cells are in nondividing conditions while other cells divided (Bernadotte et al. 2016). These nondividing cells support the proliferative potential of the tissue. When one cell exhausts its proliferative ability, other cells can be recruited. The more cells undergo irreversible cell cycle arrest, the less proliferative potential of the tissues remains. Thus, it is reasonable to determine the amount of the shortest telomeres instead of average telomere length.

The DNA damage theory of aging states that the main cause of aging-related functional decline is the accumulation of DNA mutations and damage. Mutations refer to the changes in the nucleotide sequence, including deletions, insertions, substitutions, or rearrangements of base pairs, and can lead to dysfunctional proteins. DNA damages are physical or chemical alterations in the structure of the double helix, which can cause cellular alterations and disruption of tissue homeostasis (Szilard 1959). Such events may lead to aneuploidy, gene amplification, and loss of heterozygosity and eventually to partial or full loss of gene functions, alterations in gene expression, and genome instability (Vijg 2000). In addition, the cell has a powerful repair system to counteract DNA damage. When the repair mechanisms are not sufficient enough to cope with a given level of damage, cells may undergo a wide range of phenotypic changes, from cell cycle arrest, apoptosis, or cellular senescence to malignant transformation (Erol 2011).

The concept of immunosenescence reflects age-related changes in immune responses, both cellular and serological, affecting the process of generating specific responses to foreign and self-antigens. The decline of the immune system with age is reflected in the increased susceptibility to infectious diseases, poorer response to vaccination, and increased prevalence of cancer, autoimmune, and other chronic diseases. Both innate and adaptive immune responses are affected by the aging process; however, the adaptive response seems to be more affected by the age-related changes in the immune system. Additionally, aged individuals tend to present a chronic low-grade inflammatory state that has been implicated in the pathogenesis of many age-related diseases (atherosclerosis, Alzheimer's disease, osteoporosis, and diabetes). However, some individuals arrive to advanced ages without any major health problems, referred to as healthy aging. The immune system dysfunction seems to be somehow mitigated in this population, probably due to genetic and environmental factors yet to be described.

Although the studies of the mechanisms of aging have been conducted for centuries and several theories of aging have been created, they just describe one side of the original cause, and aging is still a mysterious progress. There still is a long road to study the mechanisms of aging and develop more effective anti-aging interventions.

17.2 Survival Curves in Lifespan Experiment

The last two decades have witnessed the exploration of the area of aging research. Many approaches have been applied to discover genetic and environmental factors that regulate aging in various organisms (Guarente et al. 2007; Antebi 2007). The most important experiment to examine the effects of certain treatments on aging is the measurement of lifespan. Survival curves showing the proportion of surviving population versus time are an intuitive means of illustrating the whole lifespan of a group of organisms and remain a key component in lifespan experiment. When discussing aging, it is important to distinguish two points on survival curves. The first one is mean lifespan, which corresponds to the age at which the horizontal line for 50% survival intersects the survival curve. The second is maximum lifespan, which corresponds to the age at which the survival curves touch the age axis (0% survival). In animal experiments, maximum lifespan represents the mean lifespan of the most long-lived 10% individuals.

Survival curves are generated from a life table and are used to estimate the rate of aging in a population. The same general assumptions concerning the rate of aging that are applied to the life table can be used for the survival curve. Indeed, comparing the shape of the survival curve with the log of mortality rate demonstrates that as the nature of mortality changes, so does the shape of the survival curve. As shown in Fig. 17.2, survival curves usually have three types. Curve A is a pure exponential decay curve, whose mortality declines with age. If mortality is independent of age, the survival curve is linear (curve B). For species with death rates that increase with age, the curve is concave (curve C). Curing specific diseases such as heart disease

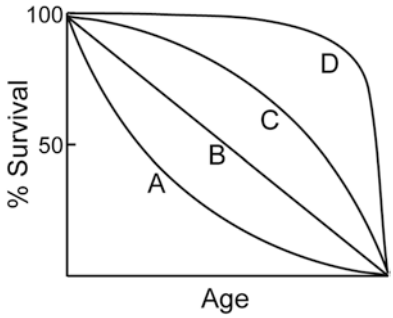

Fig. 17.2 Different types of natural survival curve. (**a**) Convex; (**b**) linear; (**c**) concave; (**d**) square

or cancer can do more than further "square" the survival curve toward curve D, with no effect on maximum lifespan.

By performing appropriate statistical analyses on survival data, we can extract a wealth of useful information. For instance, the Kaplan-Meier curve is usually used to estimate the survival longer than time *t*, and a log-rank test was usually used to determine whether experimental treatments significantly affected lifespan or not. Kaplan-Meier survival curve is defined as the probability of surviving in a given length of time while considering time in many small intervals. This estimate method is widely used in various fields. For instance, in clinical trials, the effect of an intervention is assessed by measuring the number of subjects survived or saved after certain treatment over a period of time. In the field of economics, this method is used to estimate the time of unemployment since they lose job. In industrial field, it is used to estimate the work time of certain machine. There are three assumptions used in this analysis. Firstly, the patients who are censored or continue to be followed are assumed to have the same survival prospects at any time. Secondly, the survival probability is the same through the study period. Thirdly, the events are assumed to happen at the time specified (Goel et al. 2010). The log-rank test is used to test whether the difference between control and treatment groups is significant or not, but it is unable to test the effect of other independent variables. Moreover, hazard ratio is defined as the ratio of risk occurring at any given time in one group compared with another group. To conclude, Kaplan-Meier estimate is a useful method that plays a vital role in generating evidence-based information on survival time.

17.3 Model Organisms and Anti-aging Interventions in Aging Research

Animal models used to investigate the genetic and physiological basis of aging and aging-related diseases should try to mimic the biological changes that occur with age while controlling for intrinsic and extrinsic influences. Genetic background, diet, environment, and health status can be strictly controlled in many model organisms. Yeast is measured by monitoring the number of daughter cells generated by an

individual mother cell (replicative lifespan) or by monitoring the survival of a population of nondividing cells (chronological lifespan). *C. elegans* and *Drosophila* are classic model organisms in lifespan experiments, for the reason of short lifespan and easily determined the death or alive. Nevertheless, mammalian model organisms are indispensable to understanding human aging. Although primates might be ideal in this aspect, most primates live too long, and there are ethical issues if we use primates for experiment. So mice are good models for the reason that they have shorter lifespan and can be genetically modified. Though mice differ from humans in a number of aspects, they are quite similar to humans in much of their physiology and cellular functions (Vanhooren and Liber 2012).

Various anti-aging interventions have been demonstrated to extend the lifespan of model organisms ranging from yeast to nematodes to fruit flies to rodents, with contradictory reports in rhesus monkeys (Hunt et al. 2011; Lin et al. 2014; Martin-Montalvo et al. 2013; Mattison et al. 2012). These interventions are generally classified as calorie restriction, genetic alteration, pharmaceutical administration (Verdaguer et al. 2012), and so on.

Calorie restriction, which usually refers to a 20–40% reduction in calorie intake, is the best-studied intervention for modulating aging and has been reported to prolong both mean and maximum lifespan in most organisms examined (Fontana et al. 2010; Kenyon 2005). On the contrary, feeding mice a high-calorie diet results in age-related obesity, cardiovascular diseases, and other metabolic disorders, and it shortens lifespan (Baur et al. 2006; Pearson et al. 2008; Milne et al. 2007). Although not yet definitive, results from the ongoing calorie restriction in monkeys also suggest that mortality rate in calorie restriction animals will be lower than that in control subjects. Furthermore, calorie restriction monkeys have lower body temperatures and insulin concentrations than control ones, and both of these variables are biomarkers for longevity in rodents. Recently it has been reported that longevity can be manipulated through altering macronutrient content, with mice fed a low-protein, high-carbohydrate diet having maximal lifespan (Solon-Biet et al. 2014). In addition, other feeding conditions used in long-term experiments of calorie restriction in mice are temporal restriction, intermittent fasting, and alternate day feeding. Temporal restriction means that food is provided ad libitum for a limited amount of time each day (e.g., only during the night or only during the day) and is removed thereafter. Intermittent fasting means that food is withheld for 1 or more days per week. Alternate day means that food is provided ad libitum for 24 h (feeding day) and is then withdrawn for 24 h (fasting day), which is also referred to as every other day feeding. Though the use of calorie restriction has been used successfully for centuries, the critical factors for its beneficial effects remain elusive. Calorie restriction is accomplished by restricting either how much (amount, calorie restriction) or when (timing, temporal restriction) food is provided. Calorie restriction studies have uncovered roles for molecules involved in various nutrient-sensing pathways, such as insulin/IGF-1, SIRT1, NAMPT, PGC-1α, and mTOR in regulating aging and lifespan, and those pathways are also strongly affected by the timing of food availability.

A large array of genetic alterations have been found to increase lifespan in model organisms such as yeast, nematode worms, fruit flies, and mice. For example, *SIR2* is a well-known longevity gene. Deletion of *SIR2* shortens the lifespan, and overexpression of *SIR2* extends the lifespan of various model organisms. However, the uncontrollability of genetic alteration hinders its application to human society. The development of pharmacological treatment is currently in the focus of biogerontological research.

Anti-aging drugs can be divided into three categories: the first ones are those demonstrating anti-aging effects but without any evidence yet of their ability to prolong life; the second ones are those that are suggested to extend longevity primarily because they can prevent the progression of particular aging-related disease; others are suggested to reverse the aging process itself, at least in certain environments. Although no drugs have been approved as anti-aging drugs by the Food and Drug Administration, delaying aging through anti-aging drugs has always been a cause of interest to many researchers. Many compounds can extend the lifespan of various organisms, such as silent information regulation factor 1 (SIRT1) activator, metformin, rapamycin, and other antiaging drugs. These anti-aging drugs can be classified into three general categories: natural anti-aging drugs, synthetic anti-aging drugs, and others. Natural anti-aging drugs widely existed in plants. For example, resveratrol is a kind of plant polyphenol that widely existed in plant and red wine, which can initiate longevity gene *SIR2*, inhibit tumor gene p53, block cell apoptosis, delay senescence, and prolong lifespan. Aspirin is a kind of antipyretic, analgesic, and anti-inflammatory synthetic drug. It is reported that aspirin can extend the average lifespan of worms and increase life expectancy and stress resistance. Metformin is widely used to treat type 2 diabetes and metabolic syndrome, and it can prolong the lifespan of nematodes by regulating folic acid metabolism and methionine metabolism. Metformin is also reported to act on the electron transfer chain and activate the AMPK protein kinase, thereby prolonging the lifespan of mice (Chin et al. 2014).

Until recently, numerical data from animal survival experiments were usually not shared, and the data were analyzed only in the original study. Whether these interventions extend the lifespan via universal, distinct, or overlapping patterns remains unclear. The limitation hampers method development, reanalysis of existing data by new methods, meta-analyses combining data, and systems biology approaches (Ziehm et al. 2015).

17.4 Meta-analysis of Survival Data

Meta-analysis is a statistical analysis that combines the results of multiple scientific studies. The basic principle under meta-analysis is that there is a common truth behind all conceptually similar scientific studies but which has been measured with a certain error within individual studies. The aim of meta-analysis is to use approaches from statistics to derive a pooled estimate closest to the unknown common truth based on how this error is perceived. In essence, all existing methods

yield a weighted average from the results of the individual studies, and what differs is the manner in which these weights are allocated and also the manner in which the uncertainty is computed around the point estimate thus generated. In addition to providing an estimate of the unknown common truth, meta-analysis has the capacity to contrast results from different studies and identify patterns among study results, sources of disagreement among those results, or other interesting relationships that may come to light in the context of multiple studies. A key benefit of meta-analysis is that we can derive more robust and higher statistical information than from any individual study. However, researchers must make choices which can affect the results when conducting a meta-analysis, including deciding how to search for studies, selecting studies based on a set of objective criteria, dealing with incomplete data, analyzing the data, and accounting for publication bias (Walker et al. 2008). The general steps of meta-analysis are as follows: formulation of the research question, search of literature, selection of studies based on quality criteria, decision on which dependent variables or summary measures are allowed, selection of a meta-analysis model, and examining sources of between-study heterogeneity.

Traditionally in aging research, survival data from lifespan experiments are mainly analyzed in the original study, and there have been very few studies combining survival data from multiple studies. Comparing beyond the primary study is generally limited to comparing changes in mean lifespan or similar high-level summary statistics. Various matters prevent meta-analysis of survival data, including the fact that survival data from lifespan experiments have not been publicly accessible and survival is influenced by many environmental factors. Besides, the experiment standard in different laboratories may vary a lot. So it's difficult to acquire enough data with identical conditions or develop methods that allow us to compare the difference accounting for varying additional factors. Ziehm et al. established a database SurvCurv, which is a good practice to address questions in research on aging that are beyond the scope of individual experiments. They characterize survival difference between female and male flies of different genetic *Drosophila* strains and found that females lived longer than males in *Drosophila*. Overall transgenic constructs of the UAS/GAL4 expression system which should have no effect extend lifespan significantly in the w1118 strain (Ziehm et al. 2013). Actually, published meta-analyses of survival data have mostly assessed calorie restriction (Swindell 2012; Nakagawa et al. 2012; Jensen et al. 2015). For example, it has been reported that calorie restriction significantly extends lifespan, and the proportion of protein intake was more important for lifespan extension than the degree of calorie restriction (Nakagawa et al. 2012). Simons et al. reanalyzed published survival data from 82 pairs of survival curves from dietary experiments in rats and mice by fitting Gompertz and also Gompertz-Makeham models, which separate initial mortality rate (vulnerability) from an age-dependent increase in mortality (aging rate). The researchers found that dietary restriction reduced aging rate without affecting vulnerability. The analysis results indicate that the biology underlying the life-extending effect of dietary restriction in rodents likely involves attenuated accumulation of damage, which contrasts with the acute effect of dietary restriction on mortality reported for *Drosophila* (Simons et al. 2013).

No study has demonstrated whether calorie restriction, gene manipulation, or pharmaceutical administration is superior in extending lifespan or resisting senility. This review attempted to address this question by conducting a comprehensive and comparative meta-analysis of the effect patterns of these different interventions and their corresponding mechanisms via survival curves. *C. elegans* and *Drosophila* are powerful model systems that are widely used in aging research. We developed an algorithm enabling us to combine multiple of these species from a large number of studies and to extract general trends from relevant or even contradictory results while controlling for species-specific and study-specific effects.

17.5 Anti-aging Interventions Reduce the Aging Rate Without Affecting Vulnerability in *C. elegans*, in Contrast to the Effects in *Drosophila*

Survival data from lifespan experiments can be extracted from the literature. We searched PubMed using the keywords "aging and anti-aging" + "longevity/lifespan" + "*C. elegans*/*Drosophila*" and examined the reference lists of the retrieved papers and reviews. Papers published on any date up to 2017 were included. From more than 2000 studies this search yielded, papers were selected that contained a graphical survival curve or, for some other studies, provided the complete data set from which a survival curve could be constructed. Papers were included or excluded from analysis based on several methodological criteria: (i) The lifespan experiment contained both a control group and a treatment group. (ii) Survival was reported until all animals died and was extractable from figures or tables in at least five binned time intervals. (iii) Strains of control groups that are transgenic or mutant must be excluded. (iv) We were careful not to include multiple publications of the same data set that can lead to multiple inclusions of the same data thereby biasing meta-analysis. (v) When multiple experimental groups (e.g., different concentration of medications) were available within the same study, we selected the experimental group of which the experimental protocol was most comparable to the control group. We restored survival data from graphs (using the digitize 2 package in MATLAB) extracted from the retrieved studies. We identified 284 studies that fitted our inclusion criteria, including 46 case-control pairs of survival curves of *C. elegans* and 238 pairs of *Drosophila*. To reduce error and smooth the curves, we used the Gompertz model, a common survival model, to fit the survival with maximum likelihood estimation (MLE). The Gompertz model describes the survival rate using the equation:

$$S(t) = e^{-\frac{a}{b}\left(e^{bt}-1\right)} \tag{17.1}$$

A smaller a indicates enhanced vulnerability in the aging process (i.e., the initial mortality rate), and a smaller b indicates slowing of the rate of aging (Fig. 17.3a) (Simons et al. 2013). First, we analyzed the survival parameter resulting from resveratrol treatment. Importantly, we were able to guarantee that the survival curves

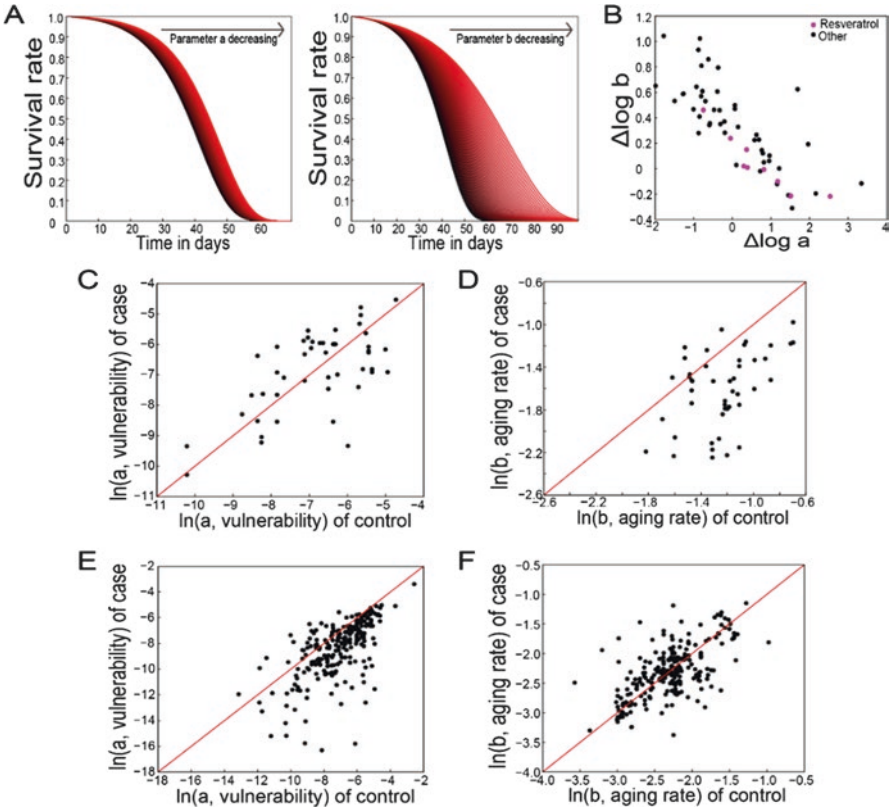

Fig. 17.3 Vulnerability ln(a) plotted against the aging rate ln(b) (Gompertz model) of controls and cases to visualize changes in the aging rate induced by different anti-aging interventions. (**a**) Schematic diagram of the changes in the survival curves with reductions in ln(a) and ln(b). (**b**) Parameter feature distribution of resveratrol in *C. elegans*. (**c**, **d**) Gompertz estimates obtained from the 46 pairs of survival curves of *C. elegans* plotted as controls against cases for a and b. (**e**, **f**) Gompertz estimates obtained from the 238 pairs of survival curves of *Drosophila* plotted as controls against the cases for a and b. When the plots lie below the x equals y line, the parameter is lower under the treatment condition (calorie restriction, medications, or genetic alteration)

for the same factors had similar parameter distributions (Fig. 17.3b). Based on the data we collected, we determined that most anti-aging interventions in *C. elegans* delayed aging by slowing the rate of aging, whereas the vulnerability to the aging process was not altered (Fig. 17.3c, d; P_a = 0.6612, P_b = 2.026e–05). Opposite results were obtained for *Drosophila* (Fig. 17.3e, f; P_a = 2.357e–06, P_b = 0.2164, respectively). Thus, the lifespan extension effects of anti-aging interventions appeared to differ in these two models. Furthermore, our analysis suggested that the aging rate could be lowered without increasing vulnerability, in contrast to the compensation law of mortality. This observation suggests that the aging rate is not a species-specific fixed property but a property that can be modified.

17.6 Meta-analysis of Survival Curves Indicated that Calorie Restriction Is the Most Reasonable Anti-aging Intervention

We found that different anti-aging interventions extend lifespan in different models. Lifespan experiments were conducted by different laboratories, so various environmental factors affected the survival of control and case cohorts which will hinder the meta-analysis of different anti-aging patterns. Different medium, temperature, strains, and sex led to significant difference even between normal control survival curves. Therefore, we have extracted some relevant parameters, which allow us to compare the shape and scale of survival curves separately. The first feature was size improvement which can be used to describe the total improvement of certain anti-aging intervention. The second feature was anti-aging type. For a population, a pattern that occurs mainly in the early lifespan could improve the demographic structure, while the pattern effects that occur in late life may bring more burdens to society. Therefore, the parallel shift mode, with large ln(size) and a ln(type) close to zero, may be the best lifespan extension method, because it improves survival throughout the whole lifespan of the population, which does not change the lifespan structure, maintaining population stability (Liang et al. 2018).

Generally speaking, anti-aging interventions of these two organisms are mainly classified into three categories: medications, genetic alterations, and calorie restriction. The differences in survival curves of these three anti-aging interventions were commented from the biological point of view. The visualized characteristic scattering of nematodes showed that size improvement due to calorie restriction and genetic alteration was slightly larger than medications. However, genetic alteration has largely increased the maximum lifespan, while calorie restriction has improved the total lifespan. That is, the mode of improvement due to calorie restriction appears to be beneficial to more roles in the group, whereas genetic alteration seems to benefit only a few long-lived individuals. Therefore, calorie restriction is more beneficial for population because more individuals live longer, contrary to genetic alteration, which allows some long-lived individuals to spend more resources to sustain their lives. Similarly, a meta-analysis of *Drosophila melanogaster* showed a genetic transitivity, although there was no significant difference in the pattern of calorie restriction and genetic alteration. Therefore, we concluded that calorie restriction and genetic alteration resulted in a larger degree of improvement in lifespan compared to medications, although the underlying mechanism is unknown. However, the effect of calorie restriction is superior to genetic alteration in *C. elegans*.

Although the lifespan extension scales of calorie restriction and genetic alteration are greater than medications. It is difficult to apply genetic alteration to humans (Testa et al. 2014; Weinert and Timiras 2003; Tosato et al. 2007) and most people would abide by such a strict calorie restriction program for it may reduce the quality of life (Anton et al. 2008; Dirks and Leeuwenburgh 2006; Redman et al. 2008). As an alternative strategy, new research focuses on calorie restriction mimetics, which is used to identify compounds that mimic calorie restriction effects without

restricting food intake (Lane et al. 2002; Ingram et al. 2004; Everitt et al. 2005; Roth et al. 2005). For example, the drugs that inhibit glycolysis (2-deoxyglucose), enhance insulin-related pathway (metformin), or activate stress signaling pathways (resveratrol) are being assessed as CR mimetics (Ingram et al. 2006). So we examined the lifespan effects of different classes of medications, which revealed some interesting patterns of differences. The results showed that though the whole improvement due to hypoglycemic agents and antioxidants was not as large as antiepileptic agents, these medications shifted the survival curves in parallel, which might be healthier in extending lifespan for a population. It has been reported that hypoglycemic agents and antioxidants can evoke similar effects on aging, health, and lifespan to those of calorie restriction (Hadley et al. 2001; Poehlman et al. 2001). So we can conclude that CR mimetics tend to be the most robust candidate among all the anti-aging medications.

Next, we compared the biological functions of those genes in a selected region (a region that has larger size improvement and better antiaging type) with background region (those other genes we collected) and then analyzed the biological pathway with GOTERM_BP_DIRECT in David (Huang et al. 2009a, b; Dennis et al. 2003). The results showed that genes that have larger size improvement and better anti-aging pattern are largely associated with the determination of adult lifespan (GO: 0008340), oxidative stress (GO: 0006979), and nutrient (GO: 0007584) categories. The determination of adult lifespan category includes the JNK pathway, insulin-signaling pathway, and target of rapamycin (*dTOR*), which are important signaling pathways in calorie restriction (Lee et al. 2010). Besides, the oxidative stress and nutritional response pathway are important pathways for calorie restriction (Merry 2004; Luo et al. 2017), suggesting the potential benefits of calorie restriction.

17.7 Conclusion

This review introduced a useful method for measuring the changes in scale and shape changes in survival curves independently. We performed three procedures to reexamine the survival curves of two classic model organisms. Meta-analysis of survival data suggested that calorie restriction and genetic alteration are more effective than medications in delaying aging. In *C. elegans*, the effect pattern of calorie restriction is superior to that of genetic alteration but similar in *Drosophila* (Fig. 17.4). Genetic alteration in mammals faces many risks, and calorie restriction, including time-restricted feeding, changes in diet composition, or calorie restriction mimetics, is a more feasible approach for humans.

Aging is an inevitable part of life. Although the mechanisms of aging have not been fully elucidated, there are several common assumptions about aging, including free radical theory, telomere theory, reduced immune function, and brain aging centralism (Harman 1981; Mikhelson and Gamaley 2012; Bernstein and Bernstein 2006). Our research shows that hypoglycemic agents and antioxidants can significantly maintain the age structure of the population and delay aging. These drugs protect cell membrane and organelles from free radical damage and can mimic the

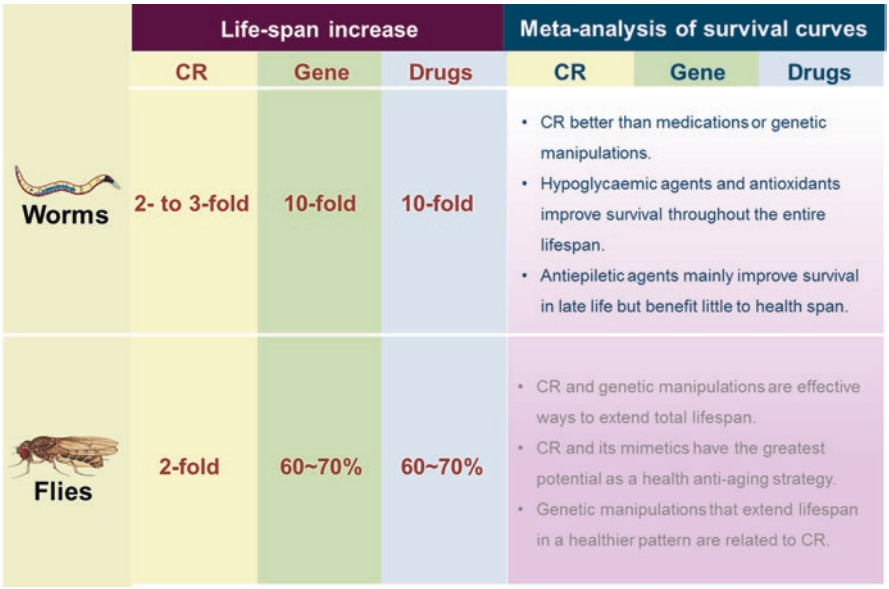

Fig. 17.4 CR and its mimetics have the greatest potential as a healthy anti-aging strategy. The effects of different anti-aging interventions exhibited fairly strong species transitivity from *C. elegans* to *Drosophila*

effects of calorie restriction. In summary, calorie restriction mimetics are key regulators for extending the lifespan of *C. elegans* and *Drosophila*.

One of the limitations of meta-analysis research is the difficulty in obtaining unbiased data. When we collected data from recently published paper, we could not control publication bias. However, as most published papers focus on the degree of improvement (e.g., log-rank test), the shape pattern analysis may be more convincing. Also, since the original survival data are usually not supplied by collected published paper, we can only use MATLAB "digitize 2" package to recover data (Hanselman and Littlefield 1997; Ingle and Proakis 2011) and cannot apply the traditional log-rank test or Cox hazard regression to analyze survival difference. Therefore, we construct a new method based on data recovery and model fitting. In addition, a SurvCurv database and online analysis platform for animal survival data have been established. All the survival records in the database were from 60 publications (Ziehm et al. 2013; Ziehm and Thornton 2013). So we checked all these publications to ensure that all the relevant survival data are included in our analysis. We used numerical survival data from *C. elegans* and *Drosophila* and specific analysis scripts to solve these questions, which were otherwise very difficult to approach. This review is currently based on a limited number of studies. Expanding these results to other organisms, such as mice or rats, requires separate inspections in future studies.

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The original version of the chapter was inadvertently published without an acknowledgement details. The acknowledgements section has now been updated with the below text:

The paragraph "Compared to evidence related to aging … with an average age of 70 years" in Sect. 9.2 of this chapter was re-used from the original work "Buford, T.W. (Dis) Trust your gut: the gut microbiome in age-related inflammation, health, and disease. Microbiome 5, 80 (2017) doi:https://doi.org/10.1186/s40168-017-0296-0".

The updated version of this chapter can be found at
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Z. Wang (ed.), *Aging and Aging-Related Diseases*, Advances in Experimental Medicine and Biology 1086, https://doi.org/10.1007/978-981-13-1117-8_18